

Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/GB05/000549

International filing date: 17 February 2005 (17.02.2005)

Document type: Certified copy of priority document

Document details: Country/Office: GB
Number: 0407807.7
Filing date: 06 April 2004 (06.04.2004)

Date of receipt at the International Bureau: 31 May 2005 (31.05.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)



World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse



PCT/GB 2005 / 0 0 0 5 4 9



INVESTOR IN PEOPLE

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

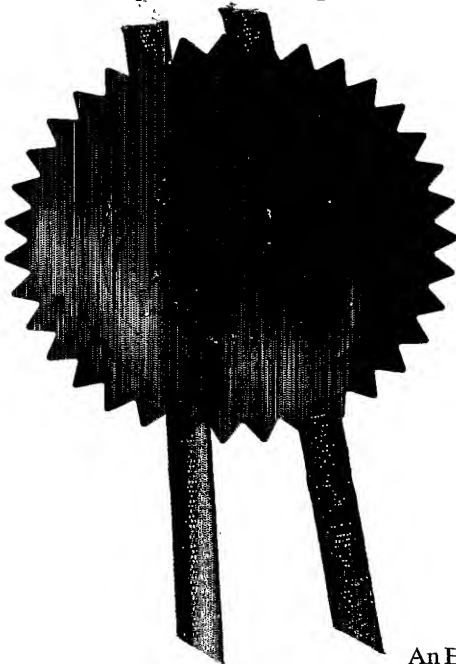
Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed

Andrew Gersey

Dated

14 March 2005



Patents Form 1/77

Patents Act 1977
(Rule 14)THE PATENT OFFICE
WM
06 APR 200406APR04 1886952-1 002973
P01/7700:0.00-0407807.7 NONE**Request for grant of a patent**

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
South Wales
NP10 8QQ

1. Your reference

P104853GB

2. Patent application number

0407807.7

6 APR 2004

3. Full name, address and postcode of the or of each applicant (*underline all surnames*)The University of York
Heslington
York
YO1 5DD
GBPatents ADP number (*if you know it*)

7511405002

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention

Desaturase enzymes

5. Name of your agent (*if you have one*)

Harrison Goddard Foote

"Address for service" in the United Kingdom to which all correspondence should be sent (*including the postcode*)31 St Saviourgate
York
YO1 8NQ
GBPatents ADP number (*if you know it*)

14571001 7914237002

6. Priority. Complete this section if you are declaring priority from one or more earlier patent applications, filed in the last 12 months.

Country

Priority application number
(*if you know it*)Date of filing
(*day / month / year*)

7. Divisionals, etc: Complete this section only if this application is a divisional application or resulted from an entitlement dispute (see note d)

Number of earlier UK application

Date of filing
(*day / month / year*)

8. Is a Patents Form 7/77 (Statement of inventorship and of right to grant of a patent) required in support of this request?

Answer YES if:

Yes

- a) any applicant named in part 3 is not an inventor, or
- b) there is an inventor who is not named as an applicant, or
- c) any named applicant is a corporate body.

Otherwise answer NO (See note d)

Patents Form 1/77



Patents Form 1/77

9. Accompanying documents: A patent application must include a description of the invention. Not counting duplicates, please enter the number of pages of each item accompanying this form:

Continuation sheets of this form

Description	23
Claim(s)	3
Abstract	1
Drawing(s)	25 only

CF

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (Patents Form 7/77)

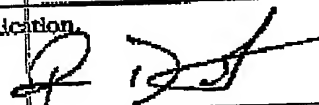
Request for a preliminary examination and search (Patents Form 9/77)

Request for a substantive examination (Patents Form 10/77)

Any other documents (please specify)

11. I/We request the grant of a patent on the basis of this application.

Signature(s)



Date

6/4/04

12. Name, daytime telephone number and e-mail address, if any, of person to contact in the United Kingdom

Rob Docherty

01904 732120

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 08459 500505.

b) Write your answers in capital letters using black ink or you may type them.

c) If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.

d) If you have answered YES in part 8, a Patents Form 7/77 will need to be filed.

e) Once you have filled in the form you must remember to sign and date it.

f) Part 7 should only be completed when a divisional application is being made under section 15(4), or when an application is being made under section 8(3), 12(6) or 37(4) following an entitlement dispute. By completing part 7 you are requesting that this application takes the same filing date as an earlier UK application. If you want the new application to have the same priority date(s) as the earlier UK application, you should also complete part 6 with the priority details.

Patents Form 1/77

Aug 03



Desaturase Enzymes

The invention relates to transgenic cells transformed with nucleic acid molecules which encode enzymes with desaturase activity and the use of these cells and enzymes in biocatalysis.

Desaturases are enzymes involved in the synthesis of long chain polyunsaturated fatty acids (PUFAs). PUFAs are fatty acids (FAs) which are essential to the normal functioning of a cell and their nutritional properties are well known. An example of a PUFA is docosahexanoic acid (DHA). DHA is a n-3 fatty acid that can be obtained directly from the diet or derived from metabolism of dietary linoleic and α -linolenic acid. The n-3 fatty acids are associated with health promoting properties. For example n-3 fatty acids have been described as anti-inflammatory, antithrombotic, antiarrhythmic, hypolipidemic and vasodilatory. As such, the role of DHA in the prevention and/or treatment of diseases such as coronary heart disease, hypertension, type II diabetes, ocular diseases, arthritis, cystic fibrosis and schizophrenia has been the focus of a great deal of medical research.

The production of PUFAs involves a consecutive series of desaturations and elongations of the fatty acyl chain to generate arachidonic acid (20:4 Δ 5,8,11,14) and docosahexaenoic acid (22:6 Δ 4,7,10,13,16,19). Several desaturases involved in this metabolic process have been isolated from marine microalgae, including *Phaeodactylum tricornutum* [5], *Euglena gracilis* [6] and *Pavlova lutheri* [7]. These membrane-bound desaturases are specific with respect to both chain length of the substrate and the double bond positions on the fatty acid. They belong to the class known as front-end fatty acid desaturases due to the fact that they introduce double bonds between the carboxy-group and pre-existing bond(s) of the fatty acid [1]. These desaturases contain a cytochrome b5 domain at their N-terminus and three histidine motifs that are important for catalytic activity [10].

Desaturase enzymes and the genes which encode them are known in the art. For example, WO03/064596 describes, amongst other things, transgenic cells transformed with omega 3 and delta 12 desaturase nucleic acid molecules and the use of these cells in the production of fatty acids. In particular the use of the omega 3 desaturase in the conversion of arachidonic acid to eicosapentaenoic acid and the use of the delta 12 desaturase in the conversion of oleic acid to linoleic acid. WO03/099216 also describes fungal desaturases and in particular transgenic plants modified to express fungal delta 15 desaturase enzymes.

- 10 Furthermore, US2003/0157144 and US2003/0167525 disclose delta 5 and delta 6 desaturase genes in the conversion of dihomoγlinolenic acid to arachidonic acid and linoleic acid to γ-linolenic acid respectively. Moreover, US2003/134400 discloses delta 4 desaturase genes which are involved in the conversion of adrenic acid to ω6-docosapentaenoic acid and in the conversion of ω3-docosapentaenoic acid to docosahexaenoic acid. These rare fatty acids are used in pharmaceutical and cosmetic compositions and can be essential nutritional fatty acids.

- Besides the common FAs 16:0, 16:1Δ9, 18:0 and 18:1Δ9 found in most living organisms, trace amounts of more unusual fatty acids can be found in a wide range of species. For instance, presence of 16:1Δ11 has been reported in several species of *Pavlova*, in the Eustigmatophyte *Nannochloropsis oculata*, and in the diatoms *Phaeodactylum tricornutum* and *Thalassiosira pseudonana* [11,12,13]. This FA accounted for a very small portion of the total FAs in these microalgae, and its specific role in the algal cells is unknown. However, this FA is a very important precursor in the synthesis of sex pheromones in insects. Sex pheromones are species-specific blends of unsaturated fatty acid (UFA) derivatives that differ in terminal functional group and in the number, position and configuration (Z or E) of the double bond(s), which are produced by various acyl-CoA desaturases [14,15]. Simple monoene Δ11 UFAs are the most prevalent precursors in the formation of major sex pheromone components in the modern *Lepidoptera* [16,17]. For instance, in the corn earworm *Helicoverpa zea*, which produces a pheromone mixture of Z11-16:Ald and

Z9-16:Ald in a 30:1 ratio, the most abundant desaturase-encoding transcript is *HzealPAQ* (also called *HzPGDs1*) which encodes a $\Delta 11$ -desaturase that does not possess a cytochrome *b5* extension, and therefore requires free cytochrome *b5* for activity. Many acyl-CoA $\Delta 11$ -desaturases with different specificities have been
5 isolated from insects [14,15], but none from other species. We describe the first characterisation of a cytochrome *b5* desaturase exhibiting $\Delta 11$ -desaturase activity.

According to an aspect of the invention there is provided a transgenic cell comprising a nucleic acid molecule which comprises a nucleic acid sequence which nucleic acid
10 molecule consists of the sequences as represented in Figures 5a, 5b, 6a, 6c, 7a, 8a, 8b, 9a, 10a, 11a, 11b, 11d or nucleic acid molecules which hybridise to these sequences, wherein said nucleic acid molecules encode a polypeptide which has desaturase activity.

15 In a preferred embodiment of the invention said hybridisation conditions are stringent hybridisation conditions.

In a preferred embodiment of the invention said nucleic acid molecule comprises a nucleic acid sequence which has at least 30% homology to the nucleic acid sequence
20 represented in Figures 5a, 5b, 6a, 6c, 7a, 8a, 8b, 9a, 10a, 11a, 11b, 11d. Preferably said homology is at least 40%, 50%, 60%, 70%, 80%, 90%, or at least 99% identity with the nucleic acid sequence represented in Figures 5a, 5b, 6a, 7a, 8a, 8b, 9a, 10a, 11a, 11b and which encodes a polypeptide which has desaturase activity.

25 The sequence of desaturase nucleic acids may be modified to produce variant enzymes with enhanced expression in cells. For example, the addition of a codon that encodes an alanine amino acid may facilitate recombinant expression in microbial systems e.g. yeast. These modifications may not be required in all expression systems but is sometimes desirable.

30

In a preferred embodiment of the invention said nucleic acid molecule comprises the nucleic acid sequence as represented in Figures 5a, 5b, 6a, 6c 7a, 8a, 8b, 9a, 10a, 11a, 11b, 11d. Preferably said nucleic acid molecule consists of the nucleic acid sequence as represented in Figures 5a, 5b, 6a, 7a, 8a, 8b, 9a, 10a, 11a, 11b.

5

In a further preferred embodiment of the invention said cell over-expresses said desaturase encoded by said nucleic acid molecule.

10 In a preferred embodiment of the invention said over-expression is at least 2-fold higher when compared to a non-transformed reference cell of the same species.

Preferably said over-expression is at least 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, or at least 10-fold when compared to a non-transformed reference cell of the same species.

15

In a preferred embodiment of the invention said nucleic acid molecule is a cDNA.

In yet a further preferred embodiment of the invention said nucleic acid molecule is a genomic DNA.

20

In a preferred embodiment of the invention said transgenic cell is transfected with a nucleic acid molecule comprising a nucleic acid sequence as represented by Figure 10a and which encodes a desaturase polypeptide wherein said polypeptide has $\Delta 11$ -desaturase activity, or a nucleic acid molecule which hybridises to the nucleic acid molecule in Figure 10a and encodes a polypeptide with $\Delta 11$ -desaturase activity.

25

In an alternative preferred embodiment of the invention said transgenic cell is transfected with a nucleic acid molecule comprising a nucleic acid sequence as represented by Figure 8a and which encodes a desaturase polypeptide wherein said polypeptide has $\Delta 6$ -desaturase activity, or a nucleic acid molecule which hybridises

30

to the nucleic acid molecule in Figure 8a and encodes a polypeptide with $\Delta 6$ -desaturase activity.

In a preferred embodiment of the invention said transgenic cell is a eukaryotic cell.

5

In an alternative preferred embodiment of the invention said cell is a prokaryotic cell.

In a further preferred embodiment of the invention said eukaryotic cell is a plant cell.

10 Plants which include a plant cell according to the invention are also provided as are seeds produced by said plants.

In a preferred embodiment of the invention said plant is selected from: corn (*Zea mays*), canola (*Brassica napus*, *Brassica rapa* ssp.), flax (*Linum usitatissimum*),
15 alfalfa (*Medicago sativa*), rice (*Oryza sativa*), rye (*Secale cereale*), sorghum (*Sorghum bicolor*, *Sorghum vulgare*), sunflower (*Helianthus annuus*), wheat (*Triticum aestivum*), soybean (*Glycine max*), tobacco (*Nicotiana tabacum*), potato (*Solanum tuberosum*), peanuts (*Arachis hypogaea*), cotton (*Gossypium hirsutum*), sweet potato (*Ipomoea batatas*), cassava (*Manihot esculenta*), coffee (*Coffea* spp.), coconut (*Cocos nucifera*),
20 pineapple (*Ananas comosus*), citrus tree (*Citrus* spp.) cocoa (*Theobroma cacao*), tea (*Camellia sinensis*), banana (*Musa* spp.), avocado (*Persea americana*), fig (*Ficus casica*), guava (*Psidium guajava*), mango (*Mangifer indica*), olive (*Olea europaea*), papaya (*Carica papaya*), cashew (*Anacardium occidentale*), macadamia (*Macadamia intergrifolia*), almond (*Prunus amygdalus*), sugar beets (*Beta vulgaris*), oats, barley,
25 vegetables and ornamentals.

Preferably, plants of the present invention are crop plants (for example, cereals and pulses, maize, wheat, potatoes, tapioca, rice, sorghum, millet, cassava, barley, pea), and other root, tuber or seed crops. Important seed crops are oil-seed rape, sugar
30 beet, maize, sunflower, soybean, sorghum, and flax (linseed). Horticultural plants to which the present invention may be applied may include lettuce, endive, and

vegetable brassicas including cabbage, broccoli, and cauliflower. The present invention may be applied in tobacco, cucurbits, carrot, strawberry, sunflower, tomato, pepper.

- 5 Grain plants that provide seeds of interest include oil-seed plants and leguminous plants. Seeds of interest include grain seeds, such as corn, wheat, barley, rice, sorghum, rye, etc. Oil-seed plants include cotton, soybean, safflower, sunflower, Brassica, maize, alfalfa, palm, coconut, etc. Leguminous plants include beans and peas. Beans include guar, locust bean, fennugreek, soybean, garden beans, cowpea,
10 mungbean, lima bean, fava bean, lentils, chickpea, etc.

According to a further aspect of the invention there is provided a seed comprising a plant cell according to the invention. Preferably said seed is from an oil seed plant.

- 15 According to a yet further aspect of the invention there is provided a reaction vessel comprising at least one polypeptide according to the invention, fatty acid substrates and co-factors wherein said vessel is adapted for the desaturation of said fatty acids substrates.

- 20 In a preferred embodiment of the invention said polypeptide is expressed by a cell according to the invention.

Preferably said cell is a eukaryotic cell, for example a yeast cell.

- 25 In an alternative preferred embodiment of the invention said cell is a prokaryotic cell.

According to a further aspect of the invention there is provided a method to desaturate a fatty acid substrate comprising the steps of:

- 30 i) providing a reaction vessel according to the invention; and
ii) growing said cells contained in said reaction vessel under conditions which allow the desaturation of at least one fatty acid substrate.

An embodiment of the invention will now be described by example only and with reference to the following tables and figures:

5 Table 1 illustrates the composition of major fatty acids in *T. pseudonana*;

Table 2 illustrates the major fatty acids of pYES and pYDESN yeast transformants with and without addition of exogenous saturated fatty acids;

10 Table 3 illustrates the $\Delta 6$ desaturase activity of TpDESI compared to that of an homologous *Phaeodactylum tricornutum* desaturase;

Figure 1 illustrates the predicted protein sequences with homology to front-end desaturases derived from the *T. pseudonana* draft genome. Sequence alignments of
15 12 putative *T. pseudonana* desaturases with other functionally characterised front-end desaturase enzymes identified three main blocks of homology that represent the functional domains of front-end acyl desaturases (A). The darker shaded box highlights the cytochrome *b5* haem-binding domain and shaded boxes indicate three histidine boxes. See Material and Methods for Genbank accession number and source
20 species of the functionally characterised enzymes. A phylogenetic tree of nine *T. pseudonana* desaturases with other enzymes was constructed (B). By removing the regions containing gaps (ambiguous alignment region), a dataset was created from an alignment originally made with clustalX. The tree was constructed from the dataset using Phylip3.5c software package and bootstrap analyses were carried out with 1000
25 replicates. Only well supported nodes (over 70%) are indicated with bootstrap values. All branches are drawn to scale as indicated by the scale bar (=0.1 substitutions/site). TpDESN sequence is 477 amino acids long (C). The cytochrome *b5* haem-binding domain is on a shaded background and the three histidine-boxes are framed;

30 Figure 2 illustrates RT-PCR expression analysis of *TpdesN*. Cells were harvested at different stages of growth for total RNA extraction and cDNA synthesis (A). PCR

was performed on cDNA derived from reverse transcribed RNA using *TpdesN* and *18s rRNA* specific primer pairs (B). PCR was carried out on undiluted (lane 1) and five-fold serial dilutions (lane 2-4) of each cDNA. The *18S rRNA* gene was used as a control of cDNA synthesis. EE: early exponential phase, LE: late exponential phase,

5 ES: early stationary phase;

Figure 3 illustrates GC analysis of FAMES from yeast transformed with the empty plasmid pYES2 or the plasmid containing *TpDESN*. Invscl yeast strain transformed with either pYES2 (A) or pYDESN (B) were induced for three days at 20°C without
10 supplementation before sampling for fatty acid analysis. I S. internal standard (17:0). The experiment was repeated three times and results of a representative experiment are shown;

Figure 4 illustrates mass spectra of DMDS FAME adducts from pYDESN
15 transformed yeast. Mass spectrum of the DMDS adduct of 16:1Δ9 FAME, present in all yeast samples (A). Mass spectrum of the DMDS adduct of 16:1Δ11 FAME, which was only found in yeast transformed with pYDESN (B). Picolinyl esters with spectra characteristic of 16:1Δ11 were also identified in these samples (data not shown); and

20 Figure 5a is the genomic nucleic acid sequence of the desaturase A from *T. pseudonana*; Figure 5b is the cDNA sequence desaturase A; Figure 5c amino acid sequence;

Figure 6a is the genomic nucleic acid sequence of desaturase B from *Thalassiosira*
25 *pseudonana*; Figure 6b is the partial amino acid sequence; Figure 6c is the cDNA sequence of desaturase B; and Figure 6d is the amino acid sequence of said cDNA sequence;

Figure 7a is the nucleic acid sequence of desaturase E from *Thalassiosira*
30 *pseudonana*; Figure 7b is the amino acid sequence;

Figure 8a is the nucleic acid sequence of desaturase I from *Thalassiosira pseudonana*; Figure 8b is the cDNA sequence; and Figure 8c is the amino acid sequence;

- 5 Figure 9a is the nucleic acid sequence of desaturase K from *Thalassiosira pseudonana*; Figure 9b is the amino acid sequence;

Figure 10a is the nucleic acid sequence of desaturase N from *Thalassiosira pseudonana*;

10

Figure 11a is the nucleic acid sequence of desaturase O from *Thalassiosira pseudonana*; Figure 11b is the cDNA sequence; Figure 11c is the amino acid sequence; Figure 11d is the nucleic acid sequence of desaturase O variant sequence from *Thalassiosira pseudonana*; and Figure 11e is the amino acid sequence of said
15 variant desaturase O;

- Figure 12A and 12B is a GC analysis of FAMES from yeast expressing TpDESI with exogenous substrates 18:2 Δ 9,12 (A) and 18:3 Δ 9,12,15 (B). New FAs produced from endogenous and exogenous substrates are underlined; Figure 12C is a GC analysis of
20 FAMES from yeast transformed with a vector only control compared to yeast transformed with TpDESI;

Figure 13 is an illustration of fatty acid synthesis pathways; and

- 25 Figure 14 is a GC analysis of FAMES from yeast expressing TpDESO.

Materials and Methods

- Identification of putative *Thalassiosira pseudonana* desaturase-coding sequences and phylogenetic analysis with other functionally characterised desaturases
30

The draft genome of the diatom *T. pseudonana* has been sequenced to approximately nine times coverage by the whole genome shotgun method. Sequence data were produced by the US Department of Energy Joint Genome Institute
5 (<http://www.jgi.doe.gov/>) and the raw sequence data were downloaded and installed on a local server. Batch tblastn searches were carried out using protein sequences of the following 13 known desaturases as query, including PIDES1 (AY332747, *Pavlova lutheri*), TFAD4 (AF489589, *Thraustochytrium* sp. ATCC 21685), TFAD5 (AF489588, *Thraustochytrium* sp. ATCC 21685), P Δ DEL5 (AY082392,
10 *Phaeodactylum tricornutum*), P Δ DEL6 (AY082393, *Phaeodactylum tricornutum*), E Δ DEL3 (AF139720, *Euglena gracilis*), E Δ DEL4 (AY278558, *Euglena gracilis*), Z Δ DEL (AF309556, *Danio rerio*), B Δ DEL6 (U79010, *Borago officinalis*), HsDEL5 (AF084558, *Homo sapiens*), HsDEL6 (AF084559, *Homo sapiens*), CeDEL6 (AF031477, *Caenorhabditis elegans*) and CeDEL5 (AF078796, *Caenorhabditis*
15 *elegans*).

All non-redundant sequences with an E value less than 0.001 were retrieved and assembled into contigs using the CAP3 sequence assembly program [18]. The contigs were translated into amino sequences in three frames in the orientation indicated by
20 tblastn result. Putative desaturase gene models were constructed manually based on sequence homology and in frame GT-AG intron boundaries were identified.

Deduced amino acid sequences of all 12 putative desaturase sequences of *T. pseudonana* were aligned with the above 13 functionally characterised desaturases
25 from other species, using ClustalX version 1.8 [19]. The alignment was then reconciled and further adjusted. Only nine near full-length *Thalassiosira* sequences were retained for further analyses.

A dataset of 250 conserved residue positions was used for construction of the
30 phylogenetic tree. Distance analysis used the program protdist of the Phylip 3.5c package with a PAM250 substitution matrix and a tree was then built from the matrix

using fitch (Fitch-Margoliash method). Bootstrap analyses were carried out with 1000 replicates using the neighbour-joining algorithm.

Cultivation of *T. pseudonana*

5

T. pseudonana (CCAP 1085/12) was obtained from the Culture Collection of Algae and Protozoa (Dunstaffnage Marine Lab., Oban, PA34 4AD, Scotland, U.K.). The growth medium used was enriched artificial seawater medium (EASW), made up in 20 l batches as described previously [4]. The cultures were grown in one litre flasks 10 at 15°C with 50 $\mu\text{E m}^{-2} \text{s}^{-1}$ constant illumination, and aeration provided by shaking the flasks at 150 rpm.

Cell density was monitored by counting cells with a haemocytometer. Nitrate concentration was determined periodically during the culture time by measuring the 15 change of the medium absorbance at 220 nm [20].

RNA extraction, cDNA synthesis and RT-PCR analysis

Total RNA was extracted from frozen cells harvested at different stages of growth 20 with an RNeasy plant mini kit (Qiagen). First strand cDNA was synthesised from three μg of DNase treated RNA using a Prostar First-strand RT-PCR kit (Stratagene). PCR was performed using undiluted and five-fold dilutions of cDNAs as followed: the reactions were heated to 95 °C for 5 min followed by 35 cycles at 95 °C for 30 s, 50 °C or 65°C (for *18S* rRNA and *IpdesN* respectively) for 30 s and 72 °C 25 for 2 min, then a single 72 °C for 10 min. As a marker for constitutive expression, the 18S rRNA gene was amplified with the primer TH18S5' (5'-GGTAACGAATTGTTAG-3') and TH18S3' (5'-GTCGGCATAGTTTATG-3'). *IpdesN* cDNA was amplified using primers DESNR2 (5'-GTGAGAGCACTAACCAAGCTT-3') and DESN2 (5'-CAATCAGTAGGCTTCGTC G-3'). Aliquots of PCR reaction were electrophoresed 30 through a 1% agarose gel. Identity of the diagnostic fragment amplified with *IpdesN*

specific primers was verified by sequencing after cloning in the pGEM-T EasyVector (Promega).

Functional characterisation of TpDES1 in yeast

5

The entire *Tpdes1* coding region was amplified from *T. pseudonana* cDNA with primers DesINB 5'- GCGGGATCCACCATGGCTGGAAAAGGAGGAGAC-3' (ORF start codon is indicated by bold type; underlined sequence is a *Bam*HI site;

10 *italic* sequence is an added alanine codon, not present in the original sequence of *Pldes1*) and DesICE 5'-GCGAAATCTTACATGGCAGGGAAATC-3' (ORF stop codon is indicated in bold type; underlined sequence is a *Eco*RI site). The Expand High Fidelity PCR system (Roche) was employed to minimise potential PCR errors. The amplified product was gel purified, restricted and cloned into the corresponding sites behind the galactose-inducible GAL1 promoter of pYES2 (Invitrogen) to yield 15 the plasmid pYDES1. This vector was transformed into *S. cerevisiae* strain Invsc1 (Invitrogen) by a lithium acetate method, and transformants were selected on minimal medium plates lacking uracil.

For functional expression, cultures were grown at 25°C in the presence of 2% (w/v) 20 raffinose and 1% (w/v) Tergitol NP-40 (Sigma). Expression of the transgene was induced when OD_{600nm} reached 0.2-0.3 by supplementing galactose to 2% (w/v). At that time, the appropriate fatty acids were added to a final concentration of 50 µM. Incubation was carried out at 25°C for three days.

25 Functional characterisation of TpDESN in yeast

Genomic DNA from *T. pseudonana* cells was extracted using the DNA isolation kit Puregene (Gentra Systems) and 100 ng was used to amplify the entire *TpdesN* coding region with primers DesNNB 5'- 30 GCGGGATCCACCATGGCTGACTTTCTCTCCGGC-3' (ORF start codon is indicated by bold type; underlined sequence is a *Bam*HI site; *italic* sequence is an

added alanine codon, not present in the original sequence of *TpdesN*) and DesNCE 5'-GCGAATTCTCAATCAGTAGGCTTCGT-3' (ORF stop codon is indicated in bold type; underlined sequence is a *EcoRI* site). The Expand High Fidelity PCR system (Roche) was employed to minimise potential PCR errors. The amplified product was gel purified, restricted with *EcoRI* and *BamHI* and cloned into the corresponding sites behind the galactose-inducible GAL1 promoter of pYES2 (Invitrogen) to yield the plasmid pYDES_N. The fidelity of the cloned PCR product was checked by sequencing. The vector pYDES_N was then transformed into *S. cerevisiae* strain Invsc1 (Invitrogen) by a lithium acetate method, and transformants were selected on minimal medium plates lacking uracil.

For the feeding experiment with PUFAs, cultures were grown at 22°C in the presence of 2% (w/v) raffinose and 1% (w/v) Tergitol NP-40 (Sigma). Expression of the transgene was induced when OD_{600nm} reached 0.2-0.3 by supplementing galactose to 2% (w/v). At that time, the appropriate fatty acids were added to a final concentration of 50 µM. Incubation was carried out at 22°C for three days and then 15°C for another three days. For the feeding experiment with saturated fatty acids, a single Invsc1 colony transformed with pYES2 (empty plasmid, control) or pYDES_N was inoculated in 10 ml of minimal media minus uracil containing 2% raffinose and grown overnight at 30°C with shaking (300 rpm). After 16-24 h, cells were collected by spinning at 4500 rpm for 10 min. After discarding the supernatant, the cell pellet was resuspended in the same medium mentioned above supplemented with 2% galactose and 1% tergitol, to obtain a cell density of 5×10⁷ cells/ml. Fifteen ml of this cell suspension were added to a 100 ml-flask with or without addition of saturated fatty acids (as mentioned in the text) at 500 µM final concentration. Desaturase induction was then carried out at 20°C with shaking (300 rpm) for three days.

Fatty acid analysis

Microalgae or yeast cells were harvested by centrifugation. Total fatty acids were extracted and transmethylated as previously described [4]. Most FAMES were

identified by comparison of retention times to a 37 FAME mix (Supelco). PUFA FAMES were also identified by comparison to a sample of standard Menhaden oil (Supelco) transmethyated as per the samples.

- 5 Dimethyl disulphide (DMDS) adducts were used to determine the double bond position in identified and unidentified monounsaturated FAMES. These were made by adding together 50 μ l DMDS (Aldrich), 100-1000 ng FAMES dissolved in 50 μ l hexane, and 5 μ l 50 mg ml⁻¹ iodine in diethyl ether. This solution was heated at 40°C for 15 h and partitioned with 200 μ l hexane and 100 μ l 5% (w/v) sodium
- 10 thiosulphate. The hexane phase was removed, dried under vacuum, reconstituted in 50 μ l fresh hexane and used for GC-MS analysis. A Trace GC 2000 (ThermoQuest) fitted with a 30 m x 0.25 mm x 0.5 μ m film thickness ZB-1 column (Phenomenex) was used to chromatograph 2 μ l DMDS adducts injected at 250°C and a 50:1 split ratio with He as carrier gas at 0.6 ml min⁻¹ in constant flow mode. The oven program
- 15 was 120°C for 1min then to 340°C at 5°C min⁻¹. Mass spectra were obtained using a GCQ (ThermoQuest) mass spectrometer operating in full scan mode over 50-500 m/z. Picolinyl esters were also made from FAMES to confirm their identities. These were obtained by adding 15 μ l freshly prepared 2:1 (v/v) 3-(hydroxymethyl)-pyridine (Aldrich): potassium *tert* butoxide 1 M solution in tetrahydrofuran (Aldrich) to 50 μ l
- 20 FAMES dissolved in dichloromethane. This solution was heated at 40°C for 30 min and partitioned with 200 μ l hexane and 100 μ l 2.5% (w/v) sodium hydrogen carbonate. The hexane phase was removed, dried under vacuum and reconstituted in 50 μ l fresh hexane. Picolinyl esters were injected and separated by GC-MS using the same conditions as for DMDS adducts; Sperling P., Zahringer U. and Heinz E.
- 25 (1998) A sphingolipid desaturase from higher plants. *J. Biol. Chem.* 273, 28590-28596; Sperling P., Libisch B., Zahringer U., Napier J.A. and Heinz E. (2001) Functional identification of a D8-sphingolipid desaturase from *Borago officinalis*. *Arch. Biochem. Biophys.* 388, 293-298; Whitney H.M., Michaelson, L.V., Sayanova, O., Pickett J.A. and Napier, J.A. (2003) Functional characterization of two
- 30 two cytochrome b5-fusion desaturases from *Anemone leveillei*: The unexpected

identification of a fatty acid $\Delta 6$ -desaturase. Planta 217, 983-992; each of which are incorporated by reference.

EXAMPLE 1

5 Identification and phylogenetic analysis of putative *T. pseudonana* desaturase sequences with other functionally characterised desaturases

Tblastn searches with 13 functionally characterised desaturases revealed 427 non-redundant raw sequences with E values less than 0.001. Twelve unique contigs were assembled after retrieving these sequences and gene models were constructed
10 manually based on sequence homology. These 12 gene contigs were arbitrarily designated *TpdesA* to *TpdesL*. All 12 showed significant sequence similarity to query sequences with 9 containing near full length open reading frames compared to other known desaturases (Fig. 1A). Interestingly, the predicted amino acid sequence of all nine *T. pseudonana* desaturases have a characteristic fused cytochrome *b5 haem*-
15 binding domain (HP[G/A]G) at their N-terminus and three histidine boxes (H[X]3-4H, H[X]2-3HH AND Q[X]2-3HH) with the replacement of the first histidine by glutamine in the third histidine box in all but two of the predicted proteins (*TpDESA* and *TpDESB*). These are common characteristics of a large subgroup of front-end acyl group desaturases [21]. These histidine-box motifs are critical for desaturase
20 activity, most likely because they serve to coordinate the diiron-oxo component of the active site. Three remaining sequences (*TpDESD*, *TpDESL* and *TpDESH*) appear to be partial, covering only the C-terminal end of desaturases, but nevertheless they do contain a typical third histidine box of the above mentioned subgroup of desaturases (Fig. 1A).

25

In order to gain insight into the relationships of these *T. pseudonana* sequences to other functionally characterised desaturases and especially algal desaturases, we constructed an unrooted phylogenetic tree using a Fitch-Margoliash method with statistical confidence measured by bootstrap analysis (Fig. 1B). Relationships of four
30 putative *T. pseudonana* desaturases are in well supported (>70% bootstrap value) subgroups with at least one functionally characterised desaturase from other species.

Both TpDESM and TpDESO grouped with PtDEL5, a $\Delta 5$ -desaturase from another diatom, *P. tricornutum* [5], suggesting these two enzymes may also have a $\Delta 5$ -desaturase activity. Similarly TpDESK is grouped with two $\Delta 4$ -desaturases TFAD4 and EgDEL4 from *Thraustochytrium* sp. ATCC21685 [22] and *E. gracilis* respectively. TpDESI grouped with PtDEL6, a $\Delta 6$ -desaturase from *P. tricornutum*. This indicates that TpDESK and TpDESI may have $\Delta 4$ and $\Delta 6$ -desaturase activities respectively. However, as enzymes with different regioselectivities are also found in a well supported subgroup (EgDEL8, CeDEL5 and CeDEL6; $\Delta 8$, $\Delta 5$ and $\Delta 6$ -desaturase respectively) and regioselectivity may even derive independently after a more recent duplication (CeDEL5 and CeDEL6) [23] predictions based on homology can be misleading and it is essential to functionally characterise each enzyme.

The remaining five *T. pseudonana* sequences fall into three separate subgroups (TpDESE; TpDESA and TpDESB; TpDESG and TpDESN) which do not group with any other known functional desaturases with high confidence. It is therefore possible that these proteins exhibit novel regioselectivity. The current study focussed on the characterisation of one of these proteins, TpDESN.

EXAMPLE 2

Temporal expression of TpDESN gene

RT-PCR analysis of *TpdesN* transcript was conducted at different stages of algal growth in order to establish if and when this gene is expressed. After RNA extraction and cDNA synthesis, *TpdesN* specific PCR products were amplified. PCR amplification of the 18S rDNA gene was performed as a control for the quantity of cDNA used during PCR reactions. Figure 2 shows that the diagnostic 519 bp cDNA amplification product expected for *TpdesN* was present at similar level at the different stages of cultivation of the microalga cells. Thus, *TpdesN* is transcriptionally active at a constitutive level during *Thalassiosira* growth, suggesting that it may encode a desaturase with a housekeeping function.

EXAMPLE 3**Functional characterisation of TpDESN in yeast**

5

The putative desaturase sequence annotated *TpdesN* was contained on a genomic DNA contig of 2580 bp on which no introns was detected. To establish the function of the protein encoded by this gene, the full-length sequence was amplified from genomic DNA. An alanine codon containing a G as the first letter was added immediately downstream of the start codon of *TpdesN* to ensure optimal translation in yeast [24]. The *TpdesN* ORF is 1434 bp long, and encodes a 477 amino acid protein TpDESN (Fig. 1C), having a molecular weight of 53.8 kDa. Analysis of the secondary structure of TpDESN using SOSUI software (<http://sosui.proteome.bio.tuat.ac.jp/sosui/frame0.html>) [25] predicted four transmembrane regions (not shown). Alignment of TpDESN with functionally characterised desaturase sequences mentioned above indicated an overall identity of 25%, with the cytochrome *b5*-like domain and the three conserved histidine-rich motif areas showing greatest homology.

10

15

The primary sequence of TpDESN exhibited typical features of front-end desaturases involved in PUFA synthesis. In order to characterise the specificity of this protein, PUFAs (18:2 Δ 9,12; 20:2 Δ 11,14; 20:3 Δ 8,11,14; 22:4 Δ 5,8,11,14; 18:3 Δ 9,12,15; 20:3 Δ 11,14,17; 20:4 Δ 8,11,14,17; 22:5 Δ 7,10,13,16,19) were first fed to the host yeast transformed with pYDESN and the vector alone (pYES2) as a control. Unexpectedly, after six days of incubation, TpDESN did not desaturate any of the supplemented PUFA substrate. Furthermore, there did not appear to be any production of 18:2 Δ 9,12 from endogenous 18:1 Δ 9. However, a significant increase was observed for a peak eluting in the range of sixteen carbon monounsaturated FAMES in the yeast transformed with pYDESN (Fig. 3). The position of the double bond in this product was determined by GC-MS analysis of FAMES derived to DMDS adducts [26] and picolinyl esters. The DMDS adduct of 16:1 Δ 9 FAME yielded two major fragments at m/z 145 and 217 (Fig. 4A). Fragmentation of the

20

25

30

increased FAME peak found in unfed or fed yeast transformed with pYDESN produced two diagnostic fragments at m/z 117 and 245 (Fig. 4B). This fragmentation pattern was indicative of an $\Delta 11$ monounsaturated sixteen carbon FAME, 16:1 $\Delta 11$, suggesting that TpDESN encoded a new $\Delta 11$ -desaturase. Small amounts of this FA have also been measured in *Thalassiosira* cells (Table 1). To further substantiate these results, yeast transformed with pYDESN and the control empty vector, pYES2, were cultivated in medium supplemented with saturated FA (14:0; 16:0; 18:0) representing potential substrates for the synthesis of the monounsaturated product. Yeast fatty acid profiles were analysed after three days of incubation at 20°C. Results in Table 2 showed that a small amount of 16:1 $\Delta 11$ (0.23% of total FAs) was detected in yeast transformed with pYES2, suggesting endogenous synthesis of this FA from 16:0. This FA accumulated at a higher level in both types of transformed yeast after feeding with 14:0, with values up to 5.84% in pYDESN transformants. A possible explanation for this increase in the pYES2 transformants is that the endogenous yeast $\Delta 9$ -desaturase was able to use additional 14:0 to produce 14:1 $\Delta 9$ that was subsequently elongated to 16:1 $\Delta 11$. Moreover, it has been reported that wild type yeast cells cultivated in media supplemented with 14:1 $\Delta 9$ synthesised 16:1 $\Delta 11$ by Elo1p-dependent carboxy terminal elongation [27]. After 18:0 supplementation, the percentage of 16:1 $\Delta 11$, of about 5% total FAs, was similar to that observed after feeding with 16:0. Presence of extra 18:0 could lead to an inhibition of the 16:0 chain elongation system, which might allow more 16:0 to be available for $\Delta 11$ -desaturation. On the other hand, 18:1 $\Delta 11$ represents 1.2% of the total FAs in transgenic yeast. No variation in its proportion was monitored under the different conditions of incubation, even after supplementation with 18:0 in pYDESN transformants. This suggests that this FA originates from elongation of 16:1 $\Delta 9$ rather than $\Delta 11$ -desaturation of 18:0.

EXAMPLE 4

30 Functional characterisation of TpDESI in yeast

To establish the function of TpDESI, the full-length cDNA was expressed in the yeast Invsc1 under the control of an inducible galactose promoter. Potential

substrates of front-end desaturases (18:2 Δ 9,12; 18:3 Δ 9,12,15; 20:3 Δ 8,11,14; 20:4 Δ 8,11,14,17; 22:4 Δ 7,10,13,16; 22:5 Δ 7,10,13,16,19) were tested. Figure 12A and 12B show that after supplementation of the medium with 18:2 Δ ^{9,12} and 18:3 Δ ^{9,12,15} respectively, and after three days of incubation, yeast cells containing pYDESI had extra fatty acids. Extra peaks observed when cells were fed with 18:2 Δ 9,12 had a retention time identical to 16:2 Δ 6,9, 18:2 Δ 6,12 and 18:3 Δ 6,9,12 (Figure 12A). Extra peaks observed when cells were fed with 18:3 Δ 9,12,15 had a retention time identical to 16:2 Δ 6,9, 18:2 Δ 6,12 and 18:4 Δ 6,9,12,15 (Figure 12B). These results demonstrate that *TpdesI* encodes a Δ 6-desaturase which can introduce double bond in exogenously fed 18:2 Δ 9,12 and 18:3 Δ 9,12,15 fatty acids, but also in endogenous 16:1 Δ 9 and 18:1 Δ 9 fatty acids. Percentages of conversion of these different substrates are given in Table 3.

Fatty acid profiling of marine microalgae had shown that *T. pseudonana* represents a good candidate to discover genes involved in the production and storage of PUFAs [4]. Analysis of the recently completed draft genome of this microalga revealed the presence of many candidate genes for elongase and desaturase activities most probably involved in catalysing different steps of the PUFA biosynthetic process. We have identified 12 possible desaturase genes, 9 of which there is sufficient sequence information to demonstrate that they exhibit typical features of front-end desaturases, i.e. a cytochrome b5 domain in the N-terminus and three histidine clusters located at highly conserved regions. Phylogenetic analysis revealed that several of the genes are closely related to a number of previously characterised front-end desaturases involved in PUFA synthesis. However, the current work highlights the fact that desaturase function, in terms of regioselectivity, cannot solely be based on prediction from primary amino acid sequence homology.

The fatty acid profile of *T. pseudonana* cells is quite diverse (Table 1), with the health beneficial EPA (20:5 Δ 5,8,11,14,17) and DHA (22:6 Δ 4,7,10,13,16,19) accounting for a large proportion. However, the number of desaturase gene sequences found in the genome was higher than we expected based on the number of different

desaturation reactions required to produce the diversity of FA in this microalga. This suggested that non-obvious desaturation reactions might also occur in the *Thalassiosira* cells. As a first step to establishing function of the many putative desaturase sequences, we focused on the *TpdesN* contig due to the fact that the sequence was full-length and intronless. A temporal expression study showed that *TpdesN* was constitutively transcribed during algal cultivation. Expression of the *TpdesN* ORF in yeast supplemented with PUFAs as potential substrates for desaturation revealed no new products. There was also no evidence of activity with the endogenous 18:1 Δ 9 which excludes the possibility that TpDESN acts as a Δ 12-desaturase. However, an increase in the peak area of a FAME eluting in the range of the sixteen carbon FAMES was identified and GC-MS based analysis revealed this to be 16:1 Δ 11 fatty acid. Small amounts of this FA are also present in wild type yeast. However, quantitative comparison of FA levels in the empty vector pYES2 and pYDESN transformants showed that proportions of 16:1 Δ 11 increased in the presence of *TpdesN* in both unfed cells and cells that had been fed different saturated FA. No other changes in either peak area or new peaks were detected in pYDESN transformants, indicating that TpDESN is specifically involved in conversion of 16:0 to 16:1 Δ 11.

The presence of small amounts of 16:1 Δ 11 have previously been reported in many microalgae, including *T. pseudonana*. However, a function for this FA in algal cells has not been established. The low quantity observed in many marine microalgae suggests that it may act as an intermediate in an as yet unidentified biosynthetic pathway. In insect cells, 16:1 Δ 11 represents an important precursor for pheromone synthesis, where it is produced by an acyl-CoA Δ 11-desaturase. Interestingly, the insect Δ 11-desaturases do not possess a cytochrome *b5* domain in their N-terminal region. This represents a major primary structure difference compared with TpDESN. The cytochrome *b5* domain is not a determinant of the substrate specificity [28]. Alignment of the desaturase domain of TpDESN with the full sequence of insect Δ 11-desaturases showed an identity of 20% (data not shown). In insect cells, Δ 11-

desaturases are more or less specific depending on the origin of the sequence and well-documented reviews exist on this subject [14,15].

In conclusion therefore, although the TpDESN primary sequence is very similar to front-end desaturases, it should not be considered a member of this family of desaturases because it acts only on 16:0. Identification of such a novel enzyme expands the functional repertoire of the membrane-bound desaturases and it should provide useful comparative information for understanding phylogenetic relationships between these enzymes. One question that remains to be answered regards whether cytochrome *b5* was independently fused to desaturases that had already acquired their different specificities, or whether an ancestral fusion protein for proximal lipid modification duplicated and subsequently evolved into different desaturases. Studies of the primary structure of the different PUFA desaturases support the fact that enzyme conversion (i.e. change of specificity) can be achieved through a relatively few structural changes [29]. The high degree of homology between the many potential front-end desaturases identified in the genome of *T. pseudonana* support this notion. Given the FA profile of *T. pseudonana* cells and the complexity of the desaturase gene family it is likely that different genes will encode $\Delta 4$, $\Delta 5$ and $\Delta 6$ desaturases. It will now be very interesting to functionally characterise these remaining putative desaturase genes and study the relationship between regioselectivity, primary amino acid sequence and phylogenetic relationship. A crystal structure for these enzymes is still not available due to technical difficulties in obtaining sufficient quantities of purified membrane-bound protein. Molecular genetic approaches involving site-directed mutagenesis have provided new insight into structure-function relationships, including for example that residues in close proximity to the histidine motifs have been found to be involved in shifting the ratio of desaturation/hydroxylation activities [30]. Detailed comparative analyses and computer modeling of these diverse desaturases from *T. pseudonana* may further guide site-directed mutagenesis studies aimed at defining key residues controlling substrate specificity and regioselectivity of the introduced double bond.

References

- [1] Napier, J.A., Sayanova, O., Sperling, P. and Heinz, E. (1999) *Trends Plant. Sci.* 4, 2-4.
- 5 [2] van de Loo, F.J., Fox, B.G. and Somerville, C.R. (1993) in: *Unusual fatty acids* (Moore, T.S., ed.) *Lipid Metabolism in Plants*, pp. 91-126.
- [3] Vazhappilly, R. and Chen, F. (1998) *J. Am. Oil Chem. Soc.* 75, 393-397.
- [4] Tonon, T., Harvey, D., Larson, T.R. and Graham, I.A. (2002) *Phytochemistry* 61, 15-24.
- 10 [5] Domergue, F., Lerchl, J., Zahring, U. and Heinz, E. (2002) *Eur. J. Biochem.* 269, 4105-4113.
- [6] Meyer, A., Cirpus, P., Ott, C., Schlecker, R., Zahring, U. and Heinz, E. (2003) *Biochemistry* 42, 9779-9788.
- [7] Tonon, T., Harvey, D., Larson, T.R. and Graham, I.A. (2003) *FEBS Lett.* 553, 15 440-444.
- [8] Lopez Alonso, D., Garcia-Maroto, F., Rodriguez-Ruiz, J., Garrido, J.A. and Vilches, M.A. (2003) *Biochem. Syst. Ecol.* 31, 1111-1124.
- [9] Pereira, S.L., Leonard, A.E. and Mukerji, P. (2003) *Prost. Leuko. Essent. Fatty Acids* 68, 97-106.
- 20 [10] Shanklin, J., Whittle, E. and Fox, D.C. (1994) *Biochemistry* 33, 12787-12794.
- [11] Volkman, J.K., Dunstan, G.A., Jeffrey, S.W. and Kearney P.S. (1991) *Phytochemistry* 30, 1855-1859.
- [12] Dunstan, G.A., Volkman, J.K., Barrett, S.M. and Garland, C.D. (1993) *J. Appl. Phycol.* 5, 71-83.
- 25 [13] Brown, M.R., Dunstan, G.A., Norwood, S.J. and Miller K.A. (1996) *J. Phycol.* 32, 64-73.
- [14] Knipple, D.C., Rosenfield, C.L., Nielsen, R., You, K.M. and Jeong, S.E. (2002) *Genetics* 162, 1737-1752.
- [15] Roelofs, W.L., Liu, W., Hao, G., Jiao, H., Rooney, A.P. and Linn Jr, C.E. (2002) 30 *Proc. Natl. Acad. Sci. USA* 99, 13621-13626.
- [16] Roelofs, W.L. and Bjostad, L. (1984) *Bioorg. Chem.* 12, 279-298.
- [17] Roelofs, W.L. and Wolf, W.A. (1988) *J. Chem. Ecol.* 14, 2019-2031.

- [18] Huang, X. and Madan, A. (1999) *Genome Res.* 9, 868-877.
- [19] Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. (1997) *Nucleic Acids Res.* 25, 4876-4882.
- [20] Collos, Y., Mornet, F., Sciandra, A., Waser, N., Larson, A. and Harrison, P.J. (1999) *J. Appl. Phycol.* 11, 179-184.
- [21] Sperling, P., Ternes, P., Zank, T.K. and Heinz, E. (2003) *Prost. Leuko. Essent. Fatty Acids* 68, 73-95.
- [22] Qiu, X., Hong, H. and MacKenzie, S.L. (2001) *J. Biol. Chem.* 276, 31561-31566.
- [23] Michaelson, L.V., Napier, J.A., Lewis, M., Griffiths, G., Lazarus, C. and Stobart A. K. (1998) *FEBS Lett.* 439, 215-218.
- [24] Kozak, M. (1987) *Nucleic Acid Res.* 15, 8125-8148.
- [25] Hirokawa, T., Seah, B.C. and Mitahu, S. (1998) *Bioinformatics* 14, 378-379.
- [26] Buser, H.R., Am, H., Guerin P. and Rauscher, S. (1983) *Anal. Chem.* 55, 818-822.
- [27] Schneider, R., Tatzer, V., Gogg, G., Leimer, B. and Kohlwein, S.D. (2000) *J. Bact.* 182, 3655-3660.
- [28] Libisch, B., Michaelson, L.V., Lewis, M.J., Shewry, P.R. and Napier, J.A. (2000) *Biochem. Biophys. Res. Commun.* 279, 779-85.
- [29] Cahoon, E.B., Lindquist, Y., Schneider, G. and Shanklin, J. (1997) *Proc. Natl. Acad. Sci. USA* 94, 4872-4877.
- [30] Brown, P., Shanklin, J., Whittle, E. and Somerville, C. (1998) *Science* 282, 1315-1317.

Claims

1. A transgenic cell comprising a nucleic acid molecule which comprises a nucleic acid sequence which nucleic acid molecule consists of the sequences represented in Figures 5a, 5b, 6a, 6c, 7a, 8a, 8b, 9a, 10a, 11a, 11b, 11d, or nucleic acid molecules which hybridise to these sequences, wherein said nucleic acid molecules encode a polypeptide which has desaturase activity.
2. A cell according to Claim 1 wherein said hybridisation conditions are stringent hybridisation conditions.
3. A cell according to Claim 1 or 2 wherein said nucleic acid molecule comprises the nucleic acid sequence as represented in Figures 5a, 5b, 6a, 6c, 7a, 8a, 8b, 9a, 10a, 11a, 11b, 11d.
4. A cell according to Claim 3 wherein said nucleic acid molecule consists of the nucleic acid sequence as represented in Figures 5a, 5b, 6a, 6c, 7a, 8a, 8b, 9a, 10a, 11a, 11b, 11d.
5. A cell according to any of Claims 1-4 wherein said cell over-expresses said desaturase encoded by said nucleic acid molecule.
6. A cell according to any of Claims 1-5 wherein said transgenic cell is transfected with a nucleic acid molecule comprising a nucleic acid sequence as represented by Figure 10a and which encodes a desaturase polypeptide wherein said polypeptide has $\Delta 11$ -desaturase activity, or a nucleic acid molecule which hybridises to the nucleic acid molecule in Figure 10a and encodes a polypeptide with $\Delta 11$ -desaturase activity.

7. A cell according to any of Claims 1-5 wherein said transgenic cell is transfected with a nucleic acid molecule comprising a nucleic acid sequence as represented by Figure 8a and which encodes a desaturase polypeptide wherein said polypeptide has $\Delta 6$ -desaturase activity, or a nucleic acid molecule which hybridises to the nucleic acid molecule in Figure 8a and encodes a polypeptide with $\Delta 6$ -desaturase activity.
8. A cell according to any of Claims 1-7 wherein said transgenic cell is a eukaryotic cell.
9. A cell according to any of Claims 1-7 wherein said cell is a prokaryotic cell.
10. A cell according to Claim 8 wherein said eukaryotic cell is a plant cell.
11. A plant comprising a cell according to Claim 8.
12. A seed comprising a cell according to Claim 9.
13. A plant or seed according to Claim 11 or 12 wherein said plant or seed is an oil seed plant.
14. A reaction vessel comprising at least one polypeptide encoded by a nucleic acid molecule wherein said nucleic acid molecule comprises a nucleic acid sequence consisting of the sequences as represented in Figures 5a, 5b, 6a, 6c, 7a, 8a, 8b, 9a, 10a, 11a, 11b, 11d, or nucleic acid molecules which hybridise to these sequences, and which encode a polypeptide which has desaturase activity; fatty acid substrates and co-factors wherein said vessel is adapted for the desaturation of said fatty acids substrates.
15. A vessel according to Claim 14 wherein said polypeptide is expressed by a cell according to the invention.

16. A vessel according to Claim 15 wherein said cell is a yeast cell.

17. A vessel according to Claim 15 wherein said cell is a prokaryotic cell.

5

18. A method to desaturate a fatty acid substrate comprising the steps of:

- i) providing a reaction vessel according to any of Claims 14-17; and
- ii) growing said cells contained in said reaction vessel under conditions which allow the desaturation of at least one fatty acid substrate.

10

15

20

25

30

ABSTRACT

We describe transgenic cells transformed with nucleic acid molecules which encode enzymes with desaturase activity and the use of these cells and enzymes in
5 biocatalysis.

10

Table 1

Composition of the major fatty acids in *T. pseudonana*

Fatty acid	Mol % of total fatty acids
14:0	11.50
16:0	17.95
16:1Δ9	19.81
16:1Δ11	0.19
16:2Δ9,12	2.47
16:3Δ6,9,12	6.68
18:0	0.47
18:1Δ7	0.26
18:1Δ9	1.50
18:1Δ11	1.52
18:2Δ9,12	2.37
18:3Δ6,9,12	0.98
18:3Δ9,12,15	0.32
18:4Δ6,9,12,15	5.72
20:0	0.44
20:3Δ8,11,14	0.26
20:4Δ5,8,11,14	2.46
20:5Δ5,8,11,14,17	17.51
22:6Δ4,7,10,13,16,19	6.64
24:0	0.49

Microalgal cells were harvested during the exponential phase of growth as described in section 2. Fatty acid were extracted and converted to their methyl esters before analysis by GC. Each peak was identified by the retention time compared to known standards. Each value represents the average of triplicate measurements within a representative culture.

Table 2

Composition of the major fatty acids of pYES2 and pYDESN yeast transformants with and without addition of exogenous saturated fatty acids

Fatty acid	Mol % of total fatty acids							
	- substrate		+ 14:0		+ 16:0		+ 18:0	
	pYES2	pYDESN	pYES2	pYDESN	pYES2	pYDESN	pYES2	pYDESN
14:0	0.78	0.52	1.12	0.96	0.76	0.58	0.76	0.70
14:1Δ9	0.22	0.13	1.20	1.29	0.23	0.14	0.23	0.20
16:0	18.40	15.04	18.37	14.62	23.85	22.09	17.22	15.09
16:1Δ9	39.73	35.55	43.39	36.24	42.24	37.03	36.24	31.67
16:1Δ11	0.23	3.27	2.36	5.84	0.22	5.57	0.25	5.84
18:0	7.37	7.34	6.61	7.23	6.36	6.60	16.72	17.47
18:1Δ9	30.19	34.32	24.44	30.24	23.89	25.19	26.07	26.58
18:1Δ11	1.20	1.21	1.35	1.30	1.19	1.12	1.08	0.96
26:0	1.89	2.63	1.16	2.29	1.26	1.70	1.43	1.50

The values given are expressed as mol % of total fatty acid methyl esters quantified by GC. All values are the means of duplicates from a typical experiment.

TABLE 3	substrate	product	% conversion	
			<i>Thalassiosira pseudonana</i>	<i>Phaeodactylum tricornutum</i>
	16:1Δ9	16:2Δ6,9	14	6
	18:1Δ9	18:2Δ6,9	18	5
	18:2Δ9,12	18:3Δ6,9,12	54	28
	18:3Δ9,12,15	18:4Δ6,9,12,15	68	27

$16:1\Delta 9 = 16:1n-7$ $16:2\Delta 6,9 = 16:2n-7$ $18:1\Delta 9 = 18:1n-9$ $18:2\Delta 6,9 = 18:2n-9$
 $18:2\Delta 9,12 = 18:2n-6$ $18:3\Delta 6,9,12 = 18:3n-6$ $18:3\Delta 9,12,15 = 18:3n-3$ $18:4\Delta 6,9,12,15 = 18:4n-3$

Both algae are Diatoms. TpDESI and Δ6-desaturase sequences are 70% identical at the amino acid level. Data for the *Phaeodactylum* enzymes have been taken from the following paper (Table 1):

Domergue, F., Lerchl, J., Zachringer, U. and Heinz, E.

Cloning and functional characterization of *Phaeodactylum tricornutum* front-end desaturases involved in eicosapentaenoic acid biosynthesis.

Eur. J. Biochem. 269 (16), 4105-4113 (2002)

Figure 1

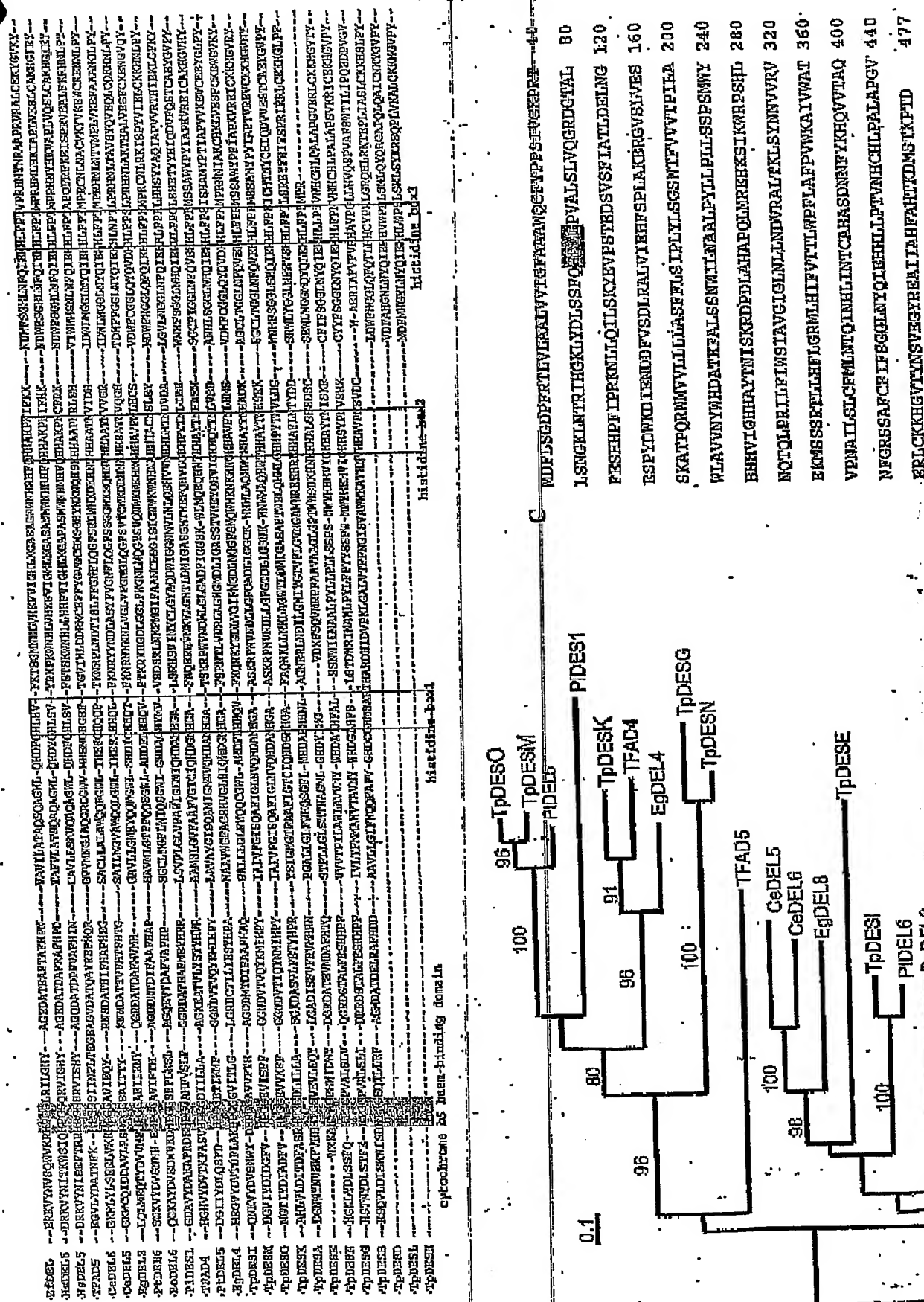


Figure 2

A

Growth phase	early exponential: EE	late exponential: LE	early stationary: ES
Incubation time	142 h	237 h	311 h
Nitrate degraded	20%	60%	100%

B

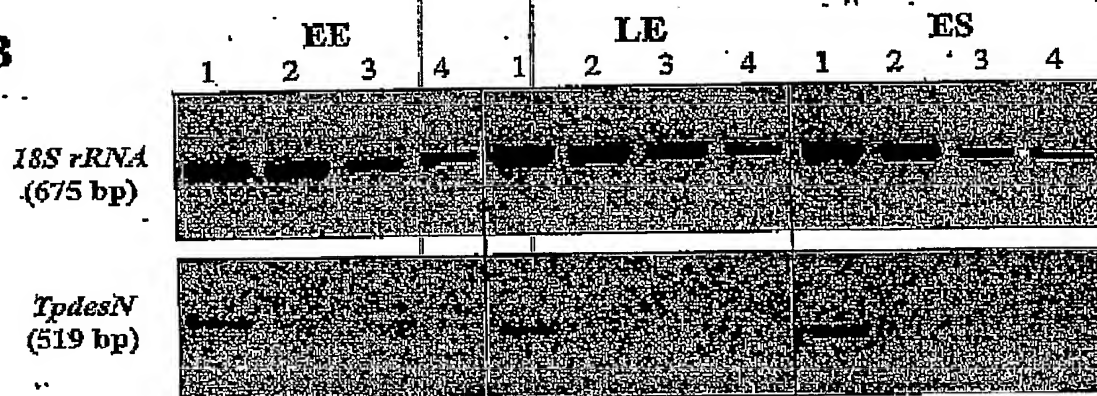


Figure 3

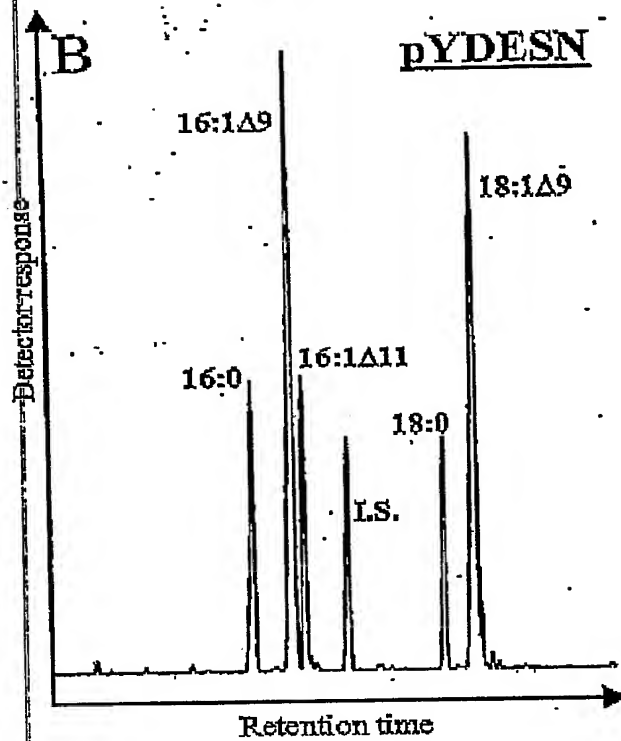
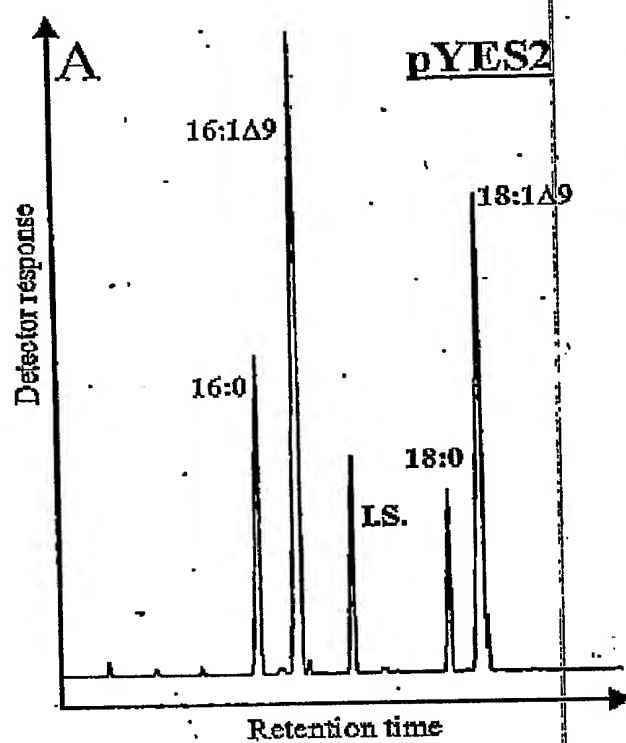


Figure 4

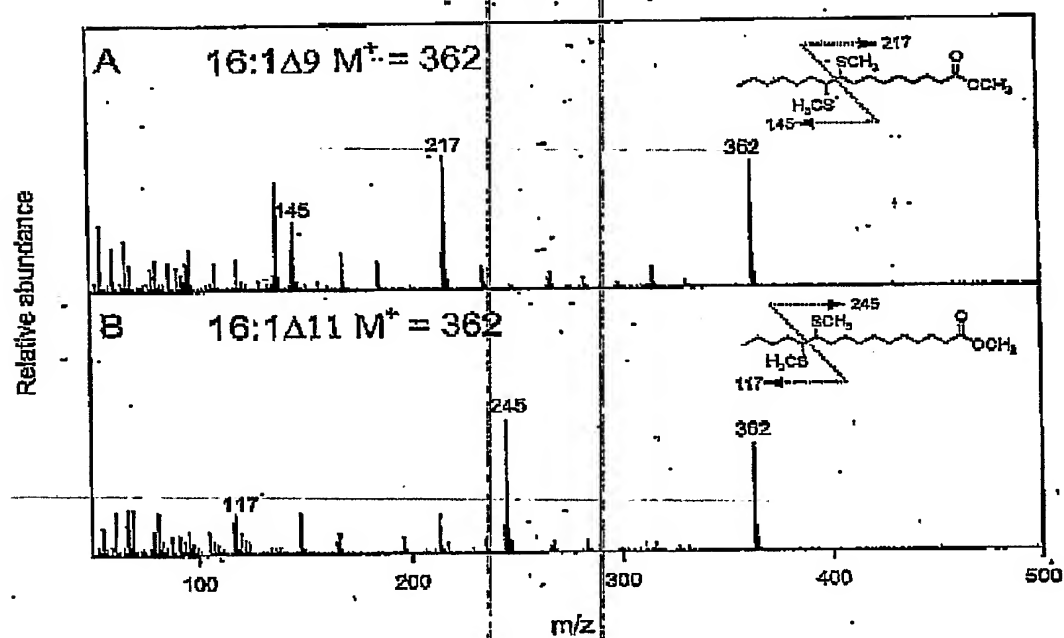


FIGURE 5a

TpDESA = *Thalassiosira pseudonana* DESATURASE A

CGGAGGGCGCACTGGAGAGGTTCCCGGAGGTTTGATGTAATTGGAGGTTGGGTCAATAC
AGATTCTGCCCTAACATTTTCGGAAATTTGGCTTCACTTTGATTCAAGCGAGGAGGCGCT
CGGCAGGAGGCGCCGTCACCTTTTTTGCATATTTGGGACTTCAATGGTTTCTACATTTTTT
CCTTTCTGGAAACCAACCGCTGTCCTTCAATTTCTCTTCCATACTCACGGATGGATCCC
CGAATATGCCACCACCAATCACCTTTGTCAATCNCAACCTCGTCATCTTTCACATTTTC
TTAGCACCATTGGCCGGTGTACCTTTCCCGGACTGCCAGTCTATGGGTCAGTATATCT
CCCACATTTGGAGAGGTTATTTGCTAAACCGTGTCAATCATACATATGATAACTGGAGAGTG
CACACCAAGAGATCAATGCTTGGAGCTAGGAGGGTGGCTATTGGCTGTGAGCGGCAGCTTT
CACTTAAGATATTTACCGCACGUCHAGTCTTCTGACAAATACAACCGATCTTCAGGTTTA
TGCAATAGCTCAAGTTGTATCAACAACAAACGTCGAGAAATCACCACAGTCTTACGAA
GATGATGCCATGAGAGCTGTTTGGCCATTGCTATGGGCGTTGAGCTGGGAACGTTGGTC
GGTTGTGCGTTAGTGTAGGAGTGCATCTTTCAGTGGAGATAACCTGACCAAACATCT
GCGATACCAACAAATCTTTCAAAACCAAGCCATATCTGATCAAAAAGCAGCTGTGACA
TCCGGCAGTACCTGCGCTGTGAGAGAGAAGGACGAAAGACGGTCTAGTACTCCTCGAT
GGCAACTGGTACCAACGTTGAAAAGTTCTGTCATCATCTCTGGAGGTTGTAAGTCTTG
GAGCAGTATCTCGGGGAGATATCTGTTTGTGTTAGAGTGTGATAGAAATCCAACT
CAAATCATGAAATATCGCAAGCCGGTACGAGCTGCCACCCAGAAAGACTTGAGGCTCTC
ACAAGCCGCGCTCAAGAGGTTTGTCTTGATATGATGACGACTTTGTTACCAATTTCCATT
GATATCGCTTCTCCAGAAATGCTTCCCAAGCCGACGCTTTTGACCTGAACTCATTGGAG
AAGGACTTCATTGACTTATATGAAGAGTTTGTGCTCAGGCTACTTCAAGCCCTCAACA
ACATGGCTACTCTGGAACACAGCGTACTGATAGTATCATCGCGTATCTGTCTATCTCA
ATGAAAGTGTCTACCACTTCTGTTTGTCTTACCTGGAGCAITGCTTGGTCTCTTTTGG
CACCAAGTGGATTCTCTATGACGATGCGGAGCACCATAATTTGGCTGGAAACGAAACGG
CTGAATGACATTTTGGGTTGATCTATGGCACTGTCTTCTTGGGTGTCAATGGCGCTTGG
TGGAGAGAGGAGCATAGACACATCATGCTTTCTCAACACTTACGATGATGAAAGTGGT
TTCAAGATCCCGAGGTTGTGTCAGCGTCACTGTAGACGACTTCAAGTTTACTTGTCTC
TGTGCTCACACATTCGATTTTATTTCACTCACTCAAGATGAGAGAGGAGCTCTGGATA
CAGAACAAGAAGTTGATTCCGTTCTTCCGTTGACGAGATCAITTCATTCTTAACAACTTT
CAGCACATCTGTTCTTCCGATCATCTTATCGTTGGCCGCGTTGGTATTGTCTGATAT
TCTACACTGACTGAGAGGAAGTTCCGTCCTTGGAGTAAGTGTCAATTTGGTATTCTTGG
AAGGAACGTGCTGATTGACTTTGATACATACTAACTGATCGGCACTTCACTACGACGAT
AGCAATACTTGGTAATGTTTGTCAATATCCTATCACTACGCAATCTTATCTCAGACGAG
TGTGCTATATCCCGTGTACATCATCGGCTCTCTTTGGCAAGCTATTCTCTCTTTGCAATT
GCTTGGGAATCACTACGTCAGGCTTGGAAATGACTCAAGGATGCCACAGAGGGAACTT
CTGCGTTTGGCAGATACTAAGCACTCAAGACTTTGATGTCCACGTTGGTCTCGGTGGCT
GTACGGAGGTCTCACTTTCACTATTCCCATCATCTATTCCCAACGTTGTCTAGAGAGTA
CTTTCACTATACATCACCAAGCATTCGGGCTGAGTGCTGCTTTAGTGTGCTACATTCA
TATCAATGATACTCAAGCTCTCATTTCCTTTCGACAGAGACTATGTGAGAGCACGGGCTT
CCGTTTATTGAGATTGCGTTTCAITGATTGCGTTGTTGGAATGCTCAACAACCTTTAACGAA
GTGAGGAAAGACTTCCGCTACGAAAGGCCACGGGAGTGTGGCTTTTCAATGTACAGGTGATCT
TAAGTGTGAGACGATATAGAGGTTGATATTTACTGTTTGTCAACAGTAGTTCGTTCA
TATGATGTAGCAACCGCAGCTTGTGGAATTAATTTAGTGTACTATGTAAGTGAAGGTT
ACGTGATCTACTCTCTGCACATCTACATCGTGTGAAGCCATTCCGTTCAAGAAATATCC
TAATGCTCTGGAACCAACAGTCTGTCCTATACCCATCATTAATCAGCCGCTCTACCCG
ATGTTGCTGTTGTTGCGGCTCTGCTGAACCCCTGCGGCGCGATAATGGCGAAGGGCA
GTGGGACACTTGATAATCTTCTTACAGATTTATGAGCTGGGTGTTTGTACCAATACCT
CCTTTATATGGTACTAATGGACCGGTGTCCATTTATGCTTGGCCGCTTTCCACCGTTT
GACCGATAGGTGGCCAAAGGCCACACAGAGGCAACCATAAAGCCGACCTTGAGGAA
ACTCAAGAAACCCGATGTTGACGATTAATAC

Figure 5b

Full length cDNA sequence: "GCT (Alanine) codon add by us to improve yeast expression

```

ATGCGCTAGACCTGTTGGGCGATTGCTATGCGCCCTTGGAGCTGGGAACCTTGGTGGTTGT
GCGTTAGTGTAGGAGTGCATCATTTTCAGTGGAGATACCTGACCAAAACATCTGCCGATA
CCAAACAAAATCTTCAAAGCAAGCCAAATATCTGATCAAAAGCACTGTGACATCCGGC
AGTACCTGCCCTGTGAGAGAGAGAGGACGAAAGACGCTGTAGTAGTCCCTGATGGCAAC
TGGTACCAACGTTGAAAAGTTCGTCCATCATCATCTGCGAGGTGTAGAGTGTGGAGCAG
TACCTCGGGGCGAGATATCTCGTTTGTGTTTAGAGTGTGGATAGAAATGCAACTCAATC
ATGAATATATCGCAGCCGCTAGGAGCTGCCAGCCGACAGAACTTCAGGCTCTCACAAGC
CGCCCTCAGAGAGTGTGTCTTGATATGATGGACGACCTTGTACCAATTCATTCATATC
GCTTCTCCAGAGATGCTTCCCAAGCCAAAGCAGTTTACCTGAGGTTCATTGAGAGGAC
TTCATTGACITATATGAGAGTGTGTGTGCTCAGGGATACCTCAAGCCCTCAACACATGG
CTCTCTGGRACACAGCCGCTACTGATTAATATCATCTCCCTTATCTCTCATCTCAATGAA
GTGCTACCAACCAACTTGTGTTTGTCTTACCTGAGCATGTGCTGGTCTCTTTTGGCAGCA
AGTGGATTCCTCATGCGATGCGGAGCACCATAATTGGCTGGAAACCAACCGCTGAAT
GACATTTTGGGTGGATCTATGGCACTGTCTTCTTGGCTGTCAATGGCCCTTGGTGGAG
GAGGACATAGAGAACATGCTTTCCTCAGACCTTACATGATGAAGTGGTTTCGAA
GATCCCGAGTGGAGAGAGGACGCTGTGATACAGAACAGAGTGTGATCCGTTCTTGGT
GACGAGATCATTCAATTTCTTACAAACCTTCAGCACATTTGTCTTCTCCGATCATCTTT
ATCGTTGGGCCGCTTGTATTTGTGTAGATTCTACACTGTCTGAGAGGAGTTCCTCTCT
TGGACATACCTGGTATGTGTTGTCTATCTCTACTACCTACGCACTCTTATCTCAGAGC
AGTCTGCTATCCCGCTGTATATCATCTCCGCTCTCTTGGGCAAGCTATTTCTCTTTGCAA
TTGCTTGGCAATCACTACGCTCAGGCTTGGATAGACTCAAGATGCCACAGAGGCAAC
TCTCTGGCTTTGGCAGATACCTAGCACTCAAGACTTTGCTATGCTCAAGCTTGGTCTCGGTTG
CTGTACGGAGGCTCTCAACTTTCACTATCCCATCATCTGTCTCCAAAGCTTGTCTAGAGAG
TACTTTACATTAATACACAGCATTGCGAGACTATGTGAGAGCACGGGCTTCCGTTT
ATTGAGATTGCTTTATGATTGCTTGTGGAATGTCAACAACTTTAAGAGTGAAG
AAAGACTTGGCTAGCAAGCCGACCGGAGTGTGGCTTCTATGATACGTTGA

```

Figure 5c

Amino acid sequence deduced from cDNA sequence

```

MARAVWALLW ALQLGLVGC ALVLGVHHS QDLTKQSAI TKSSKAKPI SDQKAAYTSC
STCAVREKAR KDGLVLLDGN WYNVEKFVHH HPGSEVYLEQ YLGADISFVE RVMHRNPQOI
MKYRKFYRAA TPSELALTS RRQEVCLDMN DDFVNSIDI ASPFMLPKPT QFDLKSFERD
FIDLXEEFVA QGYFKPSTTW LWNNTAVLIS IIALSVLSMK VLPFTSFVLE GALLGLFWHQ
SGFLMHDAEH HNLAGNERLN DILGWIYGTV FLGNGANWR ESHREHHAFL NTYDDESTFK
DPQMRREDVMI QNKELIPFFG DEIIEHLTNE OHILELPIIF IYGRVGIIVD SELTERKFKF
WILGNVCHI LLHYAILSQT SRPIPVYIIG SLMQALISIQ LIGNHYVKFW NRUNDATGKN
FCVWQILSTQ DFACFWSRW DYGLNFFYS HLEFPLSKS YPHITSPIR RLCEKHGLPF
IEIAFIDCVV GMVNNFNEVR KDEATKSGHS VAMMYT

```

TpDESB = Thalassiosira pseudonana DESATURASE B

Figure 6b

partial amino acid sequence deduced from the genomic cDNA sequence

```

MPPSIKOTLID  EEFVSEASTK  SPTTKELLPE  RKPLKRRYSF  QYSQHTFTFD  AMLTIKQSVL
DISKMSHHHF  GGEQLTLREA  GMDATDEBRA  THDDWVLEEK  LPEHVEIGVD  WTTTGABRNT
VTKDQGVSEI  IKDFRELGEH  FDRLYGTHVS  PWTYKRYAT  YEALFGCALD  LFNTEIDSTPA
HMLAAVLLGI  FWOQAFVGH  DCGHMSART  ARNDHIDVFK  GALVTFFNGI  SVANWKATHN
VHHAVPNSVD  CDPEIAHLPE  FALKHEMTH  LENKHEKRGV  EFDNLARNVT  VPQHFHWYFP
IMQVAREFNLI  IQSALFLASK  NDGHCARGCS  SKLPLALFNR  VLLVSGAGV  MHPELGAYR
IBRQPCCTYN  VTACHTTCL  LNDHLOPFRG  PGWGALSFAR  FWAFATLASR  V

```


Figure 7a

TpDESE = *Thalassiosira pseudonana* DESATURASE E

CANCTAACCGGGAAGAGGGGCTTATTTGCCAACCACAGTGATAACCTTCGG
 CTGTGACACCGGAGCAACCCGTGGGAGGCCCGCTCTGACCAGCCCTGTC
 TTTTGGAGCATCCCTCACCACACATCGCATCTCGTTCACGGGGATCAG
 TGACACAGTCTTCGTCTCATTGTTAGATGTACACGGGAAGAAGCACATCCA
 GCGGCACTCTTCATAACATCTCAGGACCGTCCAAACACGCATCACATCAT
 GATGTTCCACCGAGTCTCATCGGCATCGCGCTCACAATGGGCTGTGTCT
 CCAGTTTTCTCCTCGCCCGGTCATTCAATATTGGCCTCTCCTATGCAATCA
 TCCACCACCTTCTCGTTTCTCGACAATGATGGAAAAGTCAGAGATTTCTGA
 CAGTGTCAACAACCAAAACAGGAGATGACATCATCTTCTGAAATGCCA
 CTGCGTGGGAATGCAATGAGGAAGCTGAGTGGGTGGGAAGTTCTCTGCTGT
 GATGACGAGGAATGCCGTACTACTTTGGATGTGAGGATTCATGGCAAATG
 GTACGATCTTTCAGGTGAGTGCAAGTTGTGGTATGCAATGTTATAAGTTC
 TATTCTGTATCGGCACACAGCAATATTGTGTTGTGATCAATGTTCTAACAG
 CCATTTGTTCTCTACTTCTCAGGATGCGCAAAAGCTCACCTGCAAGG
 ACCCCACTGGATCGACTGGTACGACGGTCTGTACGCCACCGAAGTCATGG
 ACGCATTTACACCCCAAAAAGGACGTGAATGTACAGCGTCTTCCCGCG
 TCTGCCCCGAAACGGCTGCCGTTCTTGAAGCATCTGCAGCACCTTACTC
 GCACACGGAGCTTAACTTTAGGAAGTTGAGGATCAATGGGAAGTGAGG
 GGTGGTGGGAGAGGGACTTTGTCCATGAGGGAAGTGTCTGGCGATTG
 GCATCGTTGGTTACAGGAGCAGCATTGACTGCGGAGAGTGTCTCTCTCT
 TTCAACTTTCTTGTGGGATGTCTATGACGAATGCTGGATGTTGGGGC
 ATGATTATATTCTATGGTGTGTTGATAAGTTCAAGTATGAGGCCCTTT
 GCTGCCGTGGCTGCTGGTTGGGACCAACTTGGTGGAGTGATAAGCACAA
 CAAGCATCAAGCTTTGAGTGAGTCTGACTCTGTTGTTACTGCAAGTGTG
 GTTTAAGATTGAATCAATACCATCGTACTCATATCTCAACATTTCTTTC
 AATCGCAACAGCCAAACGAAATGGGAGTTGATGAAGCATTGGGACCGATC
 CATTTCTCTTTCTTTATGTTCCCGATCCAAAGTACGATTTCTCCACTTCGT
 AAGATCCAACTACATCTTCTACAGTCCCTTCTCTCTCTTTGCGCT
 CTGGCGCGTGACACCCCTTAAGGTGCGCGTAGACTCAGTTGAATCGAAAC
 GTCCCGATGCCAAGGAATGCAATTCGTGATCTGTTGGCACATTACTTCGTC
 TTGTTGACCTTCTTCCAGCTCAGGTGTGGTGGCTGCTGTCTTCTCTC
 TGGCCTCATGTCTGCACTCATTTACTCCGACACATCAGTCGGAAGAGT
 ATTTTGAGGAGTATCAGCCTGATTGGGTGACGGCTCAGTTTGAGAGCAG
 AGAAATGCTGTACGACTAATCCATTCTCTGAGTGGCTTTGGGGAGGAAT
 GCAATACAGTTGGAGCATCACTTGTTCCTTCCATGCCGAGGTAAGCAG
 CTTAATGTTTGTATCTTGTACCATTTGTTGACTTCTGTTCTCGGCTAACN
 CTGTTGGAAGCGTATGAGCCTAGCACATAATGTTGTGTATGCGACCATGA
 ACTCGATTTAAGCTTCAATACCTTACTATCATCTCAGTCCGGTGGCGGA
 TGACGTGTGTCCC

Figure 7b.

QPVPPTSSG WRKAEPAGPH WIDWYDGRDA TEVMDAFHTQ KGREMYKRLP ASAPETAAVL
 EASAAPYSQT ELNFRKLRDQ LESEGWWRD FVHEGKLLAI WASLVTGAAL TAESAPPLST
 FLLGLSMINA GWLGHDIYHG VDKFSQVMRF EAAVAAGLGF TWSDRKNKH HALSESDSCC
 YCKCELKIES IPSYSYPQHS ENRNSQKNGS

RLNQVETHTI LNILSIATAN EMGVDEDLAT DPELFPYVVD PKYDSPLRKI QHYLFYSPFS
 FLFALWRVDT LKVAVDVES KREDAKNELW YLLAHYFVLL TFFRAQWVVP AVFLGLMSA
 LIVTFTHOSE EYFEBYQPDW VTAQFESTEN AVTNPFSEN LWGMQYQLR HHLFSPMPR

Figure 8a

TpDESI = *Thalassiosira pseudonana* DESATURASE I

AAAAAAAAAANNNGGGAAGCGAGATCAATCGAGCTGGTACCATGAG
TTTCAAAAGTCAACTTCAACATTCAAGTTGTACAAAAGAGAGGGCTTCAG
ACGTGGTGAGCAAAAGCACTTCACAGGGGAATAGTAGGGGAAAACAGAA
ATATTTGGCAAATTTATCTTAGTTCCGTGATTATATCTTCAATTACTAAAG
GGAAAACAATGCAGCTCAAAAGCTACGTTTGTGTACTTCTTTGAAACAC
CTCACCCCGCGGCTTCGGCTCCGGGTCCGCCCCGCTTGCATCCTTTCTTC
CTCTCACAATTTATCTTCCAACGAGCTGATAACGTGTCAATTTACAGGGT
CAACACAATAAAACATACTAATCAACCATGGGAAAAGGAGGAGACGACG
CGCAGCCACCAAGCGTACTGGAGCAATTCATTTGGCGGACAAGCCCGAGA
AGTACACCTGGCAGGAGGTGAAGAAGCACGTGAGTCTCCGCTTGTGTTC
TGCCGTTGGATGTCTTGTCTGTTGGTTCCGATTATGCAACGAGAGTTTCT
ATTGCAACTCAATTTCAATTGTCCATCTGCAATCAACTCATCTGACCCAA
CAACTTCTGCCACCGTCCACCCATTGAGATCACCCCGACGATGCCCTGGG
TAGTCCACCAAAACAAAGTCTACGAGCTGTCCAATCGTACGACACCC
GGTGGAGCCGTGGTGTTCACCCACGCGCGAGACGACATGACGGACATCTT
CGCCGCTTCCACGCCCCAAGGCTCTCAGGCTCATGATGAAGAAGTTTTACA
TTGGAGATTTGATTCGGAGAGTGTGGAGCAAGAAGATCAAGACAGTTG
GATTTGAGAAGGGATATCGTGATTTACGGGCCAAGCTTGTCTATGATGGG
GATGTTCAAGTCAGTAAGATGTATTATGATACAAAGTCTCTGTTCAATA
TGTGATGTGGTTGGTGGCGGCTGGCCATGCTGTACTACTCGGACAGTTG
GCAATGCACATTGGATCGGCTCTCTTCTTGGATTTGCTTCTGGCAGCAGTG
TGGATGGCTTGGCGACGACTTCTTTCACCAACCAAGTCTTTAAGCAACGAA
AGTACGGAGATCTCGTTGGCATCTTTTGGGGAGATCTCATGCAAGGGTTC
TCCATGCAGTGGTGAAGAACAAGCACATGGGCCACATGCTGTCTCCAA
CTTGCACAACTCTTCTCTGGACAGTCAAGATGGTGATCCCGATATTGATA
CCATCCCACTCTCTTGGAGTCTCAAGCAGGCTCAGATTTTCAGAGAG
ATCAATAAGGGAAGGACAGTACCTTCGTCAAGTACCTATCAATTTCCA
GGCATTCACATACCTTCCCATCTCTCTTGGCTCGCATCTCTTGGTGA
ATGAATCTTCAAACTGCATTCGGACTCGGAGCTGCTCTCGGAGATGCC
AAGTTGGAGTTGGAGAAGCGTGGACTTCAGTACCCATTTTGGAGAAGCT
TGGATCACCCCTTCAATACACTTGGATGTTCTCTCTCTCTCCGATTTG
GAAGGTGGTCTCTTCCATATCCATCATGTATTTCTCACTGCCACATGC
TCTCTGGGACTTTTCTCGCATTTCTTTGGATTGGACACAACGGTAT
CTCAGTGTACGATGCCACCCGACCTGACTTCTGSCAACTCCAAGTCA
CCACTACACGTAACTCAATGGTGGACAGGGCATTCCTCAATCTTTGTG
GATTGTTCTCGGTTGGATTGCAATACCAAGTGGATCACCACCTCTTCCC
CATGTGCTTAGAAACAATATCGCGAAATGCCACAGCTTGTGGAGTCA
TCTGTAGGAGTGGGGTGTGAAGTACCATGAGGCCGATATGTGGATGGT
ACCGTGGAGTGTTCACACATCTCTCCAGGTGTGGATGATTTCTTGT
GGAGATGGTGAAGCATTTCCCTGCCATGTAAACACCTATTACAGTCCGC
AGCTTTGTCTGCTGGAGATGAATGATGCGAATCATCGTAATACTC
ATTATTAATGAACAATGTTACCTGCAAGTCTGTAGGTTTGGCTTCTGT
CCCACCGCTCTATTGTGTATTGGTGTATGATTAAGGAGATAGTCTATT
TCTACATCAGATCTCTCCATTACCCCTCGAATAGTATCCCAACAACATC
ACATCAAACTACTTGAATCTCTCTGTGCAATCCCTCCCATTTGTACATT
TACTCTCAAAGGTATATCTATTGTCCCTTTATTAATTGTTGAATATTGA
AGGGGAAGATTCCATTTTCCCTCTCTCTTCCCGATGATCTCTCACCT
CTAATACTTTTCAACAACAACAACGAACAACCGAGATCAGACAACA
ACATGGCAGAACTATCTCACCCTGCAACCGATCCCAAGCGAAGAGCTA
TTCTAGTCCATCTCCAACGATGTCTGGCTCCAGACCTCATCTGAAG
AGTGAGTGTGTATGTCTGTACTTTCCGTCTGTATTTCTCTGAGG
TGTCAAACTCAGGGTCAACAAAGCAGCTTCCCTGATCGCTAGTGGCGAG
AAGATCCGATTTCCCATCCGGAAGAAAGCTCTTGGGAAAATGTCACTT
CTTGAAAGTCCAGGGTCACTAATAATTGGGGCGGANGN

Figure 8b

GCT (Alanine) codon add by us to improve yeast expression

```

ATGGCTGGAAAAGGAGGACGACGCGAGCGGAGCTACCGAGCGTAGTGGAGCATTGAAATTC
GCGGAGAGACCCGACAGATACACTTGGCAGGAGGTGAGAGACACATCAGCCGCGACGAT
GCTGGGGTAGTCCACCAAAAGCAAGTCTACGACGCTCCCAACTGGTACGACCCACCCGGT
GGAGCCGGTGGTGTTCACCCACGCGGAGACGACATGACGACATCTTCGCGCCCTTCCAC
GCCCCAGGCTCTCAGGCTCATGATGAAGAAGTTTACATTGGAGATTGATTCCGGAGAGT
GTGGACCATAGGATCAGAGACAGTTGGATTTCCGAGAGGATATGGTGAATTTACGGGCG
AAGCTTGTGATGATGGGGATGTTCAAGTCCAGTAAGATGTATTATGCATACAGTCTCTG
TTCCATATGTGATGTGGTGGTGGGCGGCGGCGGATGCTGTAATCTCCGACAGTTTGGCA
ATGACATGTGATGTGGTGGTGGGCGGCGGCGGATGCTGTAATCTCCGACAGTTTGGCA
CAGGACTTTTCTTCCACCAAGTCTTTAAGCAACGAGTACGAGATCTGGTGGGATC
TTTTGGGGAGATCTCATGCGGGGTTCTGATGCTGCTGGGAGACACAGCAGATGGC
CACCATGCTGTTCCCAAGCTTGCACAACTCTTCTGAGACAGTCAAGATGGTGGGATC
ATTGATACCATGCGGCTCTTCTGCTGGGAGTCTCAAGCAGGCTCAGAGTTTCAAGAGATC
AATAAGGGAAAGGACAGTACCTTCTGATGATGATGATGATGATGATGATGATGATGAT
TTCCGATCTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
GGACTCGGAGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
CCACTTTTGGAGAGCTTGGAGATCAGCTTCACTACACTTGGATGTTCTGCTGCTGCTGCT
GGATTTGGAAGTGGTCTCTTCCATATTCGATCATGATGATGATGATGATGATGATGATGAT
TCCGCACTTTTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
GCCACGACGCGAGCTGATCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
GGACACGCGCATCTCCCAATTTCTTGTGGATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
GATCAGCAGCTCTTCCGATGATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
GAGTCATCTCTGAGAGTGGGTTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
GTGGAAGTGTTCACACATCTCTCCAGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT
GATTTCCCTGCCATGTAA

```

Figure 8c

Amino acid sequence deduced from the cDNA sequence

```

MAGKGGDAAA ATKRSQALKL AEKPKYKTYQ EVKCHTEFDD PWVWQNKVY DYSNWYDHPG
GAVVFTHAGD DMEDIFAAFH AQGSQAMMKK FYIGDLIPES VEHKQDQQLD FENKTRDLPA
KIVVMGNVFKS SKMYNAYECS FNMCHNLVAV AMVYSDSLA NHIGSALLLG LPWQCCGHLA
HDFLHHQVFK QKRYGDLVGI PWGDLNQGFS MOWWQNKHNG KRAVENLHNS SLDSQDGDGD
IDIMPLLANS LKQAQSFREI NKGKDSFFVK YAKFQAFY EPILLARIS KLNESFKTAF
GLGAASENAK LELEKRGLOV PLEKLGITL HYTMFVLSS GFGRWLSLFS IMYPTATCS
SGLFALVVEG LGHNGMSVYD ATRPDIWQL QVTTTNRNIIG GHGIPQFFVD WFCGGLQYQV
DHLFPMMER NNIAKCHKLV ESKKEWFKV YHADNWDGT VEVLQLSKV SDDFLVEMVK
DFFAM

```


Figure 9a

TpDESK = *Thalassiosira pseudonana* DESATURASE K

TATGTCCACCCCCCTGGTTTGTCCACCTCTCTCTTCGATCTTGGGACC
CGGGTCTCAGTTTTCGAGAGCTCTCAAGGGGGCCATAGTAGAGGACTT
GATCTGTTTGTGATACCTGAGGTGCAACCAATTTTGGGGCTAACGCCA
CTTTTCGTAACTCCACCAGGTACGACTGATTTGTGCCGTAGATATCTCT
GATACCTCTATGCCAAGCCGATCAATCGAATGATTGTACTGTAGCA
GGATAAGCAGATGGATAGGTGGGGGATCTTCATGTGACAAAGAGCAAGAG
AGAGAGTATGTCTCGCGAGGGGTGGATAGGTTCAGACACAGCGGATGAC
AGATTGTACATTATCTTCCCTCCAAGACTTTACCAAGGCACGTCACCTCTG
ATTAGAATCTTACATACACGTGCAATTAATAGTGGACAATAAATGACAAGT
GAGGACCCCCAGTGGACCAATTTCTGCGCCAGTGGTGGTCCGCTGTGGGT
TGAATGAACCGACGACGACGAACACACACCCCTCCAGCATCCACCGCACAGC
ACAATACACCAATATGTGCAACCGCAACCTTCCAGCATCCACCGCACAGC
TCAAGTCCACCTCGAAGCCCCAGGAGCAACATGAGCATCGCACCATCTCC
AAGTCCGAGCTCCGCGCACCGAACCCTCCACTCCAAACAACAACACAGC
CCACTCCACTCCGCGCACCGAACCCTCCACTCCAAACAACAACACAGC
CACACCTAGTCTCTGACATTAACCGACTTTGCGTCCCGCATCCAGGGGA
GACCTCATCTCTCTGCTTCCGCGCAAGAGCGCTCGGTGCTGTTGAAC
ATACCATCCACGTTGGAGTTCCGACCTCTCTCATTTCAAAAGCTGCAGATTG
GAGTGTATGGAGGAGGAGGCGTTTCCGGGATTCGTTTTCAGTTGGAGTGA
TCTGACTTTTATCTGTTGTTGAAGAGGAGGTTTGTGGAGCGGTTGGAGGA
GAGGGGTTTGGCGAGGAGGCGATCGAAGAGATTGATCAAGGCTTTGT
TCTTGTGTTGGATTTTGGTACTGTTTGAACAAGATGTATACTACGTG
GATATTGATCAGTACGGTATTGCCATTGCCATTCTATTGGAATGGGAAC
CTTTGCGGCATTTCATCGGCACTGTATTCAACACGATGGAAATCACGGTG
CATTCGCTCAGAACAAGTTACTCAACAAGTTGGCTGGGTGGACGTTGGAT
ATGATTGGGTGCGAGTGCCTTTACGTGGGAGCTTCAGCACATGCTGGGGCA
TCATCCATATACGAATGTGTTGGATGGGGTGGAGGAGGAGGAGGAGGA
GGGGGAGGAGATGTTGCTTTGGAAGAAAGGATCAGGTGAGACGAGATGAC
AGAGAGAGAGAGAGTCTATTCTGTGAAGTCTGATGTCATGTGTGGAT
TGAGCGACACAACCTTAACGCATTGCATTTCACCTTCAACTCGCCGACAG
CAATCAGATCCAGACGATTTCTCTCTCTCTCTCTCATGAGAATGCATCC
CCTCCATACAACCTCATGGTATCATAAATACCAACACCTCTACGCTCCAC
CCCTCTTTGCATTGATGACACTTCCCAAATATTCTCAACAGATTTTGAA
GTTGCCACATCCGACGATTAATCATATATGATGCGAATGTACGTTATGG
TTGCGATATGGAATGTGATGAGGTTTGGGCTATGAAGTCAATACGATGG
GATATATGATGGGATTAACCAATCTACTTTTCATGGAGTACTGAGCGAGTT
GGATTGTTTGTATTGGGCAATTTGGCGTGTGGAGAGTTGTTGGCGAGCAT
GTTTATTGTGAATCAGCTCAATTCAGGCTGTGAGTTATGGAACGAAGGATT
TGGTTGGTGGTGGAGTCATGTAGATGAGAAGAGATTGTCAAGCCAACG
AAGCAACAGCAATAACCAACAGAGAGAGGAGAGAGGAACTCGGAGGAGGCATTGAA
CCGTTCCATTCAACGACTGGGAGCAGTCAATGCCAGACCTCCGTGAAT
TGGTCTCCAGGCTCAGGTTCTGGAATCACTTTCTGGGGGACTCTCTCA
TCAGATTGAGCATCACTTGTTCCTCCAGCATTTGTCTATACAACTACTGTC
ATATCCAGGATGTTGTGGAGAGTACGTGTGCTGAGTACGGAGTTCGGTAT
CAGAGTGCAGAGTAATTTGTTTGTGCTTATGGAAGATGATTAGTCATTT
GAAGTTTTTGGGTAAGCCAACTGTGAGTAGGTGTTAGGTATTGAGAGGT
GTCCAGTTGTCTCATTTCTTTAAATAAAGCGGTGAATAGTATTTCGAAA
ACAAGGTTGTCTAATACCACTCTCTTGTATTGATTGCTGCGTGCACACAT
CTCCGTGAGGAGTTTGACCTCACTCATTCTAACTTGAATGTCTCTTTTG
CGCTGCTGAGCTTGGACGAATACACTCCGNCAGAGAGACTGCAATTGGTA
ATGCAGAGGAAGAGGATATACTGTATGAGTCCGAAGAAATCGATGACGG
CGGTGAGGTGGTGTACATCACTTGTGAGGACCAACCTGGAACCGCATCTC
TGAAGAGGTCCATACCTAAACATTTGAGGGGTCTTGGGAGCAAACCTTAG
CAGAGATTGAATGCTCCATTCGGTATTTGTTCTTCTGTGCCANTTTGATA
AGGAACAGCAACCAACACACCCGGG

Figure 9b

MCNGNLPAST AQLKSTSKPQ QQHEERTISK SELAQHNTPK SAWCAVHSTP ATDPSSHNNK
QHAHLVLDIT DFASRHPGGD LILLASCKDA SVLFETKPR GVPTSLIQKL QIGVMESEAF
RDSFYSWTDS DFYTVLKRRV VERLEBEGLA RRGSKBIWIK ALFLLVGFY CLYKMYTSD
IDQYGAIAAY SIGMGTFAAF IGTGICQDCN HQAFAQNKLL NKLAGWTLDL IGASAFWEL
QHMLGHHPYT NVLDGVREER KERGEDVALE BKQVRRDDR ERESLFV

QESD PDVFSSEPLM RMHPLHTTSW
YHKYQHLVAP PLFALMTLAK VFQQDFEVAT SGRLYHIDAN VRXGSVWVM RFWAMKVITM
GYDMGLPIYF HGVLRGVGLF VIGHLACSEL LATMFIVNEV IEGVSYGTD LVGGASHVDE
KKIVKPTTVL GDTPEKTR EALRSNSNNN KKKEKNSVP SVPFNDWAAV QCQTSVNWSP
GSWFVNHPSQ GLSHQTEHL FPSICHTNYC HIQOVVESTC AEYGVYPQSE SNLFVAYGKM
ISHLKFLGKA KCE

Figure 10aTpDESN = Thalassiosira pseudonana. DESATURASE N

ATGGACTTTCTCTCCGGCGATCCT
TTCCGGACACTCGTCCTTGCAGCACTTGTGTGTCATCGGATTTGCTGGGGC
GTGGCAATGCTTCTACCCGCCGAGCATCGTCGGCAAGCTTCGTACATTAA
GCAATGGTAAACTCAATACGAGAATCCATGCGAAATGTACGACCTCTCA
TCGTTTCAGCATCCAGGAGGCCCCGTGGCTCTTTCTCTTGTTCAGGTGG
CGACGGAAACAGCTCTATTTGAGTCACACCATCCCTTCATACCTCGAAAGA
ATCTACTTCAGATCCTCTCCAAGTACGAGTTCCGTGCACTGAAGACTCT
GTTTCCTTCATCGCCACCCTAGACGAACTCAATGGTGAATCTCCGTACGA
TTGGAAGGACATTGAAAATGATGATTTTCTATCTGACCTACGAGCTCTCG
TAATTGAGCACTTTTCTCCTCTCGCCAAAGGAAAGGGGAGTTTCACTCGTT
GAGTCGTGGAAGGCAACACCTCAGCGGTGGATGGTGGTTCTACTGCTCCT
TGGCTCGTTCTTCCCTCAGCATCCCATTTATATTGAGTGGTTCTGTTGACTT
TCGTTGTCTCACTCCCATCCTTGGCTTGGGCTGGCGGTGTCAATTACTGG
CAGGATGCTACTCACTTTGCAATTGACGCAACTGGATTTTGAATGCTGC
GCTCCCATATCTCTCCCTCTCCTATCGAGTCCGTCAATGTGCTATCATC
ATCACGTCATTGGACATCAGGCATACACCAACATTTGCAAAAGAGATCCA
CATCTTCTCAGCGCTCCACAACCTCATGAGGGAACACAGAGTATCAATG
GAGACCATCTCACTTAANTCAAACACAGCTTCCGCGGATTCTCTTCATCT
GGTCGATTCAGTCGGTATTGGCTTGAACCTACTGAAAGAGCTGAGAGCA
CTAACCAAGCTTTTATACAAACACGTTGTTCCGGTGGAGAGATGTATC
GTCGCGAACATTACTCCATTTCCTTGGACGTATGTTCCACATCTTTGTGA
CTACACITTTGGCCCTTTTTTGGCGTTTCCGGTGTGGAAAGGCCATCGTTTGG
GGCACTGTACCGAATGCCATACTGAGTTTGTGCTTCATGCTGAATACGCA
AATCAATCACCTCATCAACACGTTGTGCACATGCTTCCGATAACAACCTTT
ACAAGCATCAAGTTGTAACTGCTCAGAACTTTGGCCGATCAAGTGCCCTTT
TGCTTCTATCTTCTCGGGAGGTCTCAACTACCAATTGAACATCATTGTT
GCCGACGGTGAACCAATTGCCATTGCCCAGCTTTGGCCCCGGGTGTAGAGC
GTTTGTGTAAGAAACACGGGGTGACATACAACTCTGTTGAAGGATACAGA
GAGGCCATCATTGCACACTTTGCACATACCAAGATATGTGACGGAAGCC
TACTGATTGA

Figure 11a *Thalassiosira pseudonana* DESATURASE C

ANNCTCTCCACCCNGCCAGCTCTTTTCAGGTCCACCGGAGATACACACTTC
TTCCACCAACCTTCCTCCTCCATACGATCGGAAGAAAGAGGAGATTATC
TTGACTTCTTGACCGAGGAGTGGGATGAAAAGAACTTGAGTGGGTAAAGGG
CTGATTTTCCCTGAGAAGGAGAAGTCACTGGNACGAAGTTCAITGGAGITT
TGTGGCAACCCCTATTGAGACGTTGCTTGGTGGAGGAAGGTAGCGAGGTTG
AGCATGCAAAACAGAATGGTATAAATCACTAGATGTCACTCCCAATGACA
AGTAGGAATAGCAATGACGAGATGGTGTACAGATCTTAGAGATGGAGAGA
TTAAGCGAATGGCTGGATGATTAGGATATGCAATGCAAACTGTATAGAT
TCTTGTCTAATAGACTTTGTAGACAACGTCCGTCTGCAAAAAGGACAATA
CTAATTAATATRAAACCGACTCGGAGAGAACATGACATGGCAAGTTGTCA
CTATGGAAATTCACTACGTCCCTTGACAGGAAGCTCACTGGCCCTCGGCGA
AGAAGACAAAACAAAACCGAGCCCTCACATTTCACTCTGTACAGTTTCATAG
TCAACACCCACCAATACGATGCCCCCAGCCCGAATATETEEEGCATCCGC
AACCGCATCCCCACCAAAACAGGTACCGTTCGCTCTGCGGACAACAACGA
CCCCGCCACCCCAATCCGTCCGAACCCCTCAATCTCTCAAGGGCAACGAGG
TCCTCATCAACGGGACAAATTTATGACATTGCTGACTTTGTCCATCCTGGA
GGAGAGGTTGTCAAGTTCTTTGGTGGGATGATGTTACTATTCAATATAA
TATGATTTCATCCGTATCATACCGGGAACATCTCGCAAGATGAAGGCTG
TTGGAAAGGTTGTAGATTGGCAGTCCGAGTCACTTTGAATGGTGCACACG
TTGACGTTGTTGTTGTGTCATTTCTTCTTTGCAATTGATATCCAACGGA
CCCTACACACACCTCTTCTGTTACCATAGCTACAGTTCCACACCCCTTTG
AACGAGAGATCAAAATCGAAGTGTTCAGATCGTACCTCGCCGGCGGTGAG
TTCCGCACAAACAGGCTACTTCTCTCGTGCCTTTTCTACATCGCTCTCTT
CTTCACCATGCAATACACTTTTCCGACATGCAACACCTTCACACCTACG
ATCACTGGTATCAGAGTGGTGATTTCATCGCAATTGTTGTTGGTATTCA
CAGGCAATTCAATTGGGTTGAATGTCCAGCAAGATGCCAATCACCGAGCTGC
CACTAAGCCCTCCCTGGGTGAATGACTTGTTCGGATTGGAAACGGATTGA
TTGGATCTAACAAATGGAATTGGATGGCAAGCATTTGACTCATCACGCT
TACACTAACCATAGTGAGAGGATCCCGATAGCTTCAGCTCGGAACCTAT
GTTTGCATTCATGACTATCCCATTTGACACCCGAAAGAAAGTGGTGGC
ATAGGTTCCAGGGAGGGTACTTCTCTTCATGCTTGACTTTACTGGCTC
TCGACTGTATTCAATCCGCAATTCATTGATCTTCTGCAACGTTGGGGCTCA
GTACCTCGGAATTCAAATGGAGATGATTTCATTGTCAAGAGGAGCAAGT
ACGCCGTTGCATTGAGGATGATGTACATTTACTTGAACATTGTCAAGCCCC
TTCATGAACAATGGTTTGAAGCTGGTCTACCTTTGGAATCATCATGTTGAT
GGGAATCAGCGAGAGTCTCACTCTCAGTGTGCTCTCTCGTTGTCTCACA
ACTTCATCAATTCGGATCGTGATCCTACCGCTGACTTCAAAAAGACCGGA
GAACAAGCTGTCTGGTTCAAGTCCGAGGTGGAGACTTCGCTACCTATGG
GGGTTTTATTTCCGGATGTCTTACGGGAGGACTCAACTTTCAAGTGGAAAC
ATCATCTCTTTCCCCGTATGAGCAGTGTGTTGTTATCTTACATGACCT
ACGGTTCGTGAGGTTTGAAGAAGCAGGGGTGAACCTACGCTTATTATCC
TTGGATTGGGCAGAAATTTGGTATCAACATTCAAATACATGCATCGCGCTG
GTAGTCCAGCCAACCTGGGAGCTCAAGCCGTTGTCTGGAAGTGCTAAAGT
TTAGTTGTAATGATTGTGCGGAGGTGCTGCTGCTGCTCAACTAATGTTAG
GAGTGCATGTTAAAAGCCCTTCTTTGTGTTTGTGTTCTTCTGATTCAGTA
TATCAGTTTTCGATATGTTGCTTGTAACTTCCTCCACTTGCACTCAAAAC
AAATCTAGCATAACATTTCTCATCCCGAGTCATGTGATGAACGACTCATT
ACGCAATGCTCTCTCATAACCCGAAACAACTCGACCGCTTCATACTC
TAATCGTCCATCTTTGGCAGCTGCAATCCAGCCCTTGCAGCAGCTCTCTT
ACTCAACTCCATCGGACTCAACTTCTGATCTGCCCCCGCATCAATCTCAT
GCAACCGTGCCTCTCTACCAATCTGCTTTAACAATCCAGTAATCATAG
GCGATTCCACCTAGTACGTTTGTCTGCTGGGAGAGACTGATGCCGATGC
TTTGTATTGTGATATACTGTCTGCTGGTGGCGCATCGATGCTCCGNTGTG
GTTGAGACTGTGCATTGGATGCTGCTGTGAACAGTGGGTGCAGTGTAGC
GGAGGTGCTGTTCTGAACTGAGGAGATGCCCGCAACTGATAGGGGGTG
GTGCAAGGCTATAAATTTTCCGAGCGAGTCCATTGCTCTGCTCTCCCCA
TATGTCCGGCGAGGGCGAAGCGCGAAGGGGAAGCCCAAGGCCAATACAA
CAGAAAGTTTAAATGAAGGACGTAATTCCTACACAGTCCAGTGGCGAAGT
TACAAC

Figure 11b

GCT (Alanine) codon add by us to improve yeast expression

ATGCTCQCCCCCAACGCCGATATCTCCCGCATCCGCAACCGCATCCCCACCAAAACAGGT
 ACCGTTGCTCTGCGGACAACAACGACCCCGCCACCCCAATCCGTCGGAACCCCTCAATCT
 CTCAGGEGCAACGAGGTCTGTCATCAACGGCAGCAATTATGACATTGCTGACTTTGTCCAT
 CCTGGAGGAGAGGTTGTCAAGTTCTTTGGTGGGAATGATGTTACTATTTCAGTATAATATG
 ATTCATCCGTATCATACGGGGAAACATCTGAGCAAGATGAAGGCTGTTGGAAAGGTTGTA
 GATTGGCAGTCCGACTACAAGTTTCGACACCCCTTTSAACGAGAGATCAAATCAGAAGTG
 TTCAAGATCGTACGTCGCGGCGGTGAGTTTCGGCACAACAGGCTACTTCCTCCGTGCTTT
 TTCTACATCGCTCTCTTCTTACCATGCAATACACTTTCGCCACATGCAACACCTTCACC
 ACCTACGATCACTGCTATCAGAGTGGTGATTCATCGCAATTGTGTTTGGTATTTTCACAG
 GCATTCAATTGGCTTGAATGTCCAGCACGATGCCAATCAGGAGCTGCCAGTAAGCGTCCC
 TGGGTGAATGACTTGTGTTGGGATTTTGGAAACGGATTGATTGGATCTAACAAATGGAATTGG
 ATGGCACAGCAATTGGACTCATCAGCTTACACTAACCATAGTCAGAAGGATCCCGATAGC
 TTCAGCTCGGAACCTATGTTTGCATTCAATGACTATCCATTGGACACCCGAAGAGAAAG
 TGGTGGCATAGGTTCCAGGGAGGGTACTTCTCTTCATGCTTGGACTTTACTGGCTCCCG
 ACTGATTTCAATCCGCAATTCATTGATCTTCCTCAACGTCGGGCTCAGTACGTCGGAATT
 CAAATGGAGAATGATTTTCATTGTCAAGAGGAGGAAGTACGCCGTTGCATTGAGGATGATG
 TACATTTACTTTGAACATTGTTCAGCCCCCTTCATGAACCAATGCTTTGAGCTGCTTACCTTT
 GGAATCATCATGTTGATGGGAATCAGCGAGAGTCTCACTCTCAGTGTGCTCTTCTCGGTTG
 TCTCACTAATTCATCAATTCGGATCGTGATCTCTACGCTGACTTCAAAAAGACCCGAGAA
 CAAGTGTGCTGCTTCAAGTCCGAGGTGGAGACTTCTCTACCTATGGGGGTTTTATTTC
 GGATGTCTTACGGGAGGACTCAACTTTCAAGTGGAAATCATCTCTTTCCCGGTATGAGC
 AGTCTTGGTATCTTACATTCACCTACGTTCTGTTGAGGTTTGAAGAAGCACCGGATG
 AGCTACGCTTATTATCTTGGATTGGGCAGAAATTTGCTATCAACATTCAAATACATGCAT
 CGCGCTGGTAGTGGAGCCAACCTGGGAGCTCAAGCCGTTGCTGGAAGTGCCTAA

Figure 11c Amino acid sequence deduced from cDNA sequence

MAFPNADISR	IRNRIFTKTG	TVASADNNDP	ATQSVRLKS	IKGNEUVING	TIYDIADFVH
FGGEVVKFPG	GNDVTIQNM	IEFYRTGKHL	ERKCAVSKVY	DFQSDYKEDT	PFERBIKSEV
FKIVRRGRREI	GTIGYFLRAI	FYIALFFIMQ	YTFATCTFTT	TYDHWYQSGV	PIAIVFGISQ
ARIQNLVQHD	ANEGAASKRP	NVNDLLGFGT	DLTSSNIGNW	MAQHWTHHAY	THISEKDFDS
FSSBPMTAFN	DYPIGHPRK	WWRFPQGGYF	LFMLGLYWL	TUYNPQFIDL	RQRGAQYVGI
QMDNDFIVKR	RKYVALEMM	YIYLNIVSPF	MNNGLSWSTF	GLIMLNGISE	SITLSVLFLSL
SHNFINSRD	PTADEKKTGE	QVCNFKSQVE	TSSTYGGELIS	GLITGGLNFO	VEHHLFERMS
SAWYFYLAFT	VREVCKRHGM	SYAYYFWIGQ	NLVSTYKIMH	PAGCGANNEL	KPLGGSA

Figure 11d

ATGGCTCCCCCAACGCCGATATCTCCCGCATCCGCAACCGCATCCGCAACCAACAGGT
 AGCTCTGCCCCGACAAACGACGCCGCCACCCCAATCCGTCGGAACCTTCAATCTCTCAAG
 GGCAACGAGCTGGTCATCAACGGGCACAAATTTATGACATGGTGAATTTCTCCATCCGGA
 GGAGAGGTTTCAAGTTCTTTGGTGGGAATGATGTTTACATTCAGTATAATATGATTCAT
 CGGTATCATACCGGGAAACATCTGGGACAAGATGAGGCTGTTGGAAAGGTTGTAGATTGG
 CAGTCGGACTACAGTTCCGACACCCCTTTGACGAGAGATCAATCAGAGTCTTTCAG
 ATCGTACGTCGCGGGGGTGAAGTTCCGCAACAGAGGCTACTTCTCCCTGCTTTTCTAG
 ATCGCTCTCTCTTCTTACCATGTCATACACTTTTCCGCAATGCAACGCTTCACCCCTAC
 GATCACTGGTATCAGATGGTGTATTCATCGCAATTTGTTTGGTATTTACAGGCAATTC
 ATTGGGTTGATATCTCCGACAGATGCCAATCAACGGAGCTGCCACTACCGTCCCTGGGTG
 AATGACTTGTGAGATTGGCAACGGATTGATTGGATCTAACAAATGCAATTGGATGGCA
 CAGCATTCGACTCATCAGCTTACACTAACCATAGTGGAGGATCCGATAGCTTCAG
 CTGGGAACGATGTTTGCATTCAATGACTATCCGATTGGACACCCGAGAGAAAGTGGT
 GGCATAGGTTCCAGGGAGGCTACTTCTTCTATGCTTGGACTTTAGTGGCTCTCGACTG
 TATTCAATCCGCAATTCATTGATCTTCCGTCAACGTEGGCTCAGTACGTCGGAATTCAG
 TGGGAATGATTTTATTGTTCAAGAGGAGGAAGTACGCCCTTGCATTGAGGATGATGTACH
 TTTACTTGAACATTTGTCAGGCCCTTCATGAACAATGGGTTGAGCTGCTTACCTTTGGAA
 TCATCATGTTGATGGGAATCAGCGAGAGTCTCACTCTCAGTGTGCTCTTCTGTTGTTCTC
 ACAACCTCATCAATTCGGATCGTGATCCCTACGGCTGAGTTCAAAAGACCGGAGAACAG
 TGTGCTGGTTCAAGTCCGAGGTGGAGACTTCGTCTAGCTATGCGGGTTTATTTCGGAT
 GTCTTACGGGAGACTCACTTTTCAAGTTCGAACATCATCTCTTTCCCGTATGACAGTG
 CTGGGTATCCTTACATTGACCTACGCTTCCGTGAGTTTGCAGAGACACCGCGGTGAAT
 ACGCTTATTATCTCTGATTCGGGAGAAATTTGGTATCACTTTCAATATCATGCAATCGCG
 CTGGTATGGAGCCAACTGGGAGCTCAAGCCCTTCTCTGGAAGTGCCTAA

Figure 11e

MADENADISE	IRNRIPTKTS	TSADNNDPAT	QSVRTLSLK	GNEVVINGTI	YDIADFVRFG
GEVVKFPFGN	DVTIQYNNIH	PYHTGKHLEK	MKAVGVVDW	QSDYKFTTFF	EREIKSEVFX
IYRGRFEGT	TCYFLRAFFY	LALFETMQYT	FATCTTFITY	DEWYQSGVFI	AIVFGISQAF
IGLVQHDAN	HGAASKRPFV	NDLLGFQTDI	IGSNKNNWA	QWTHHAYTN	HSEKDEDSFE
SEMFARNDY	PIGHEPKRWW	HREQGGXFLP	MLGLYWLSIV	FNPQFIDLRQ	RCAGYVGIQM
ENDFIYKRRK	YAVALRMYYI	YLNIIVSFFMN	NGLSWSTFBI	IMLMGISBSL	TLSVLFSLSK
NLINSRDRPT	ADFKKIGEQV	CNFKSQVETS	STYGGTISCC	LTGCLNFQVE	HHLFFRMSSA
WYPYIAPTFR	EVCKICGVNY	AXXPWIGQNL	VETPKYMHKA	GSGANNELKP	LQSSA

Figure 12A

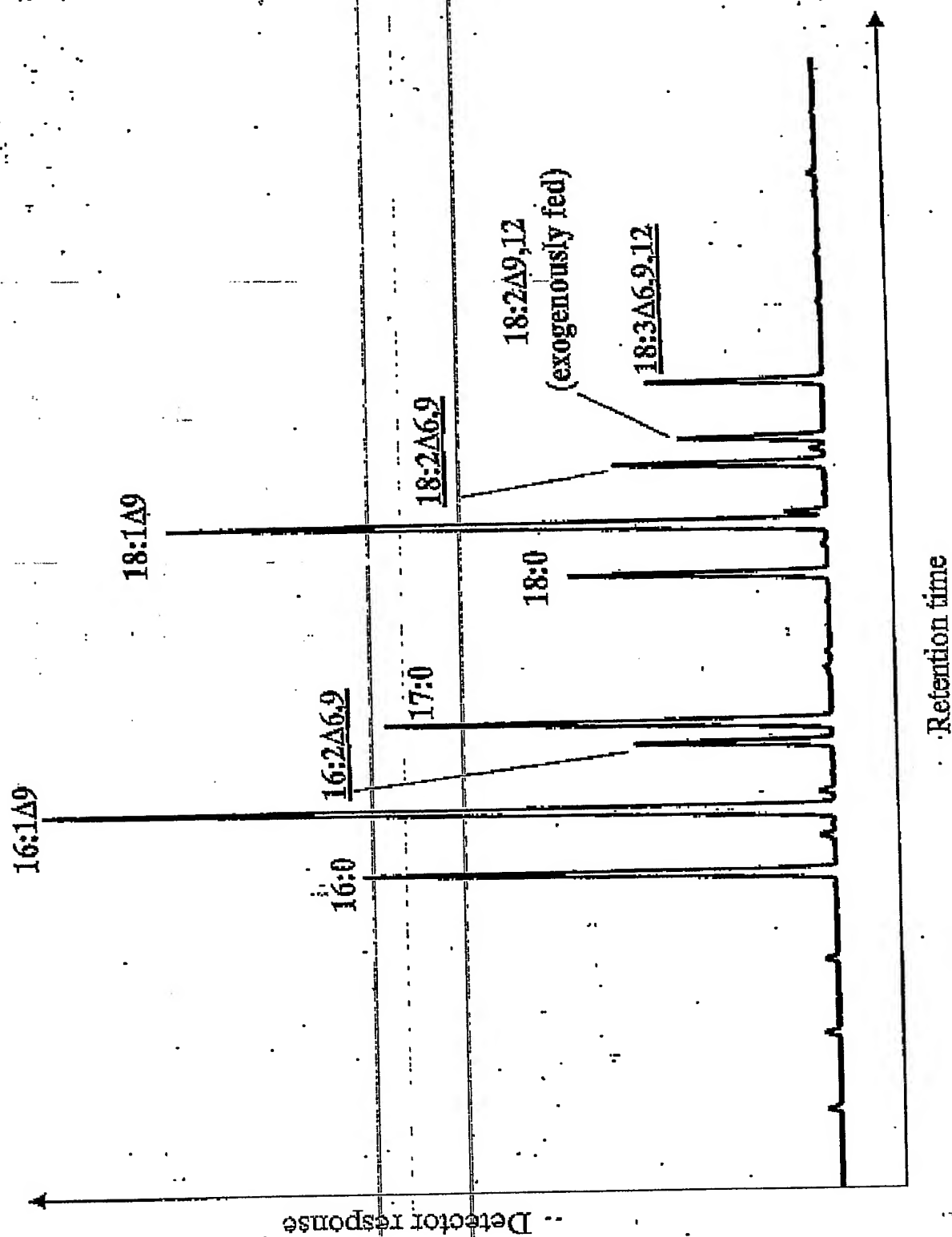


Figure 12B

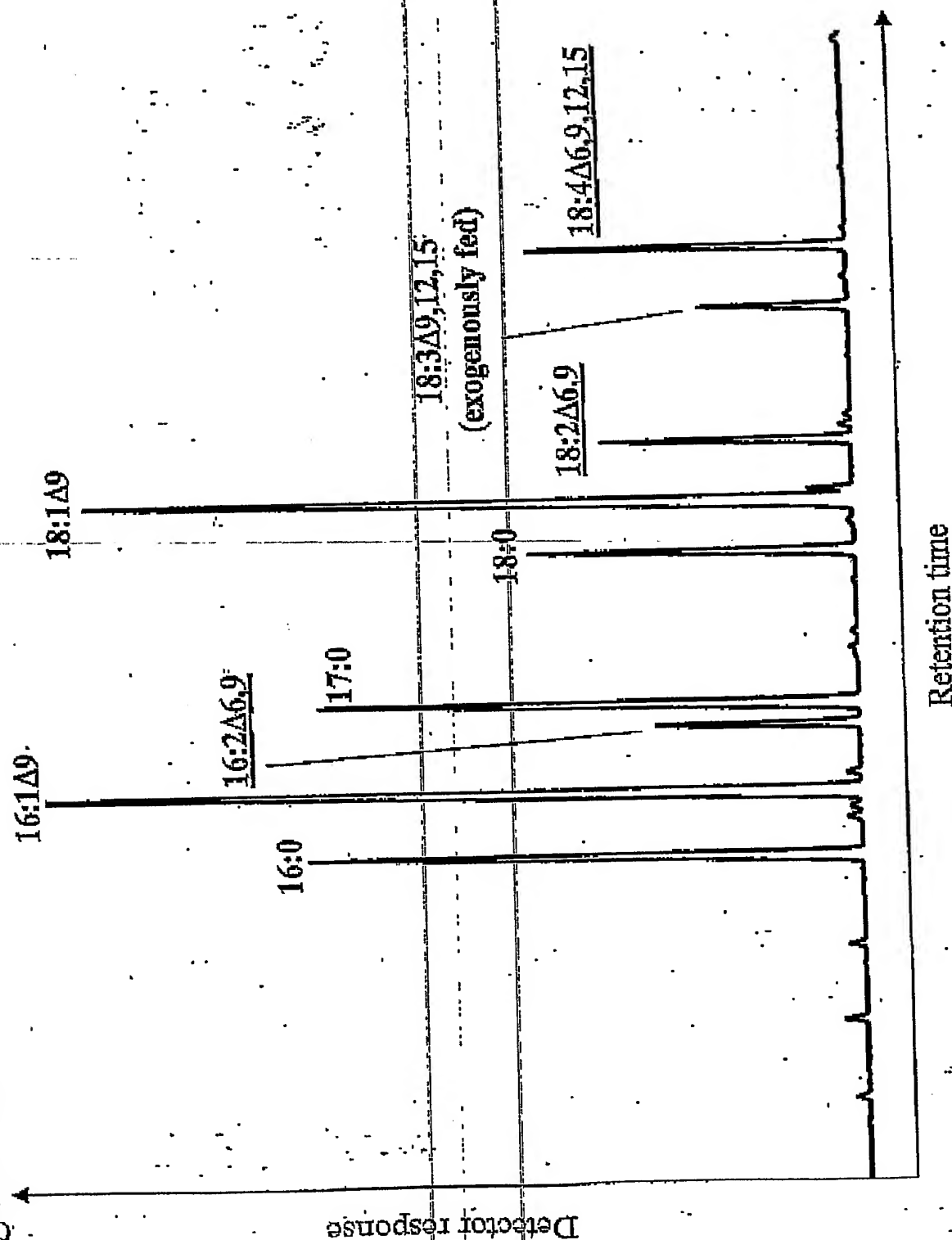


Figure 13

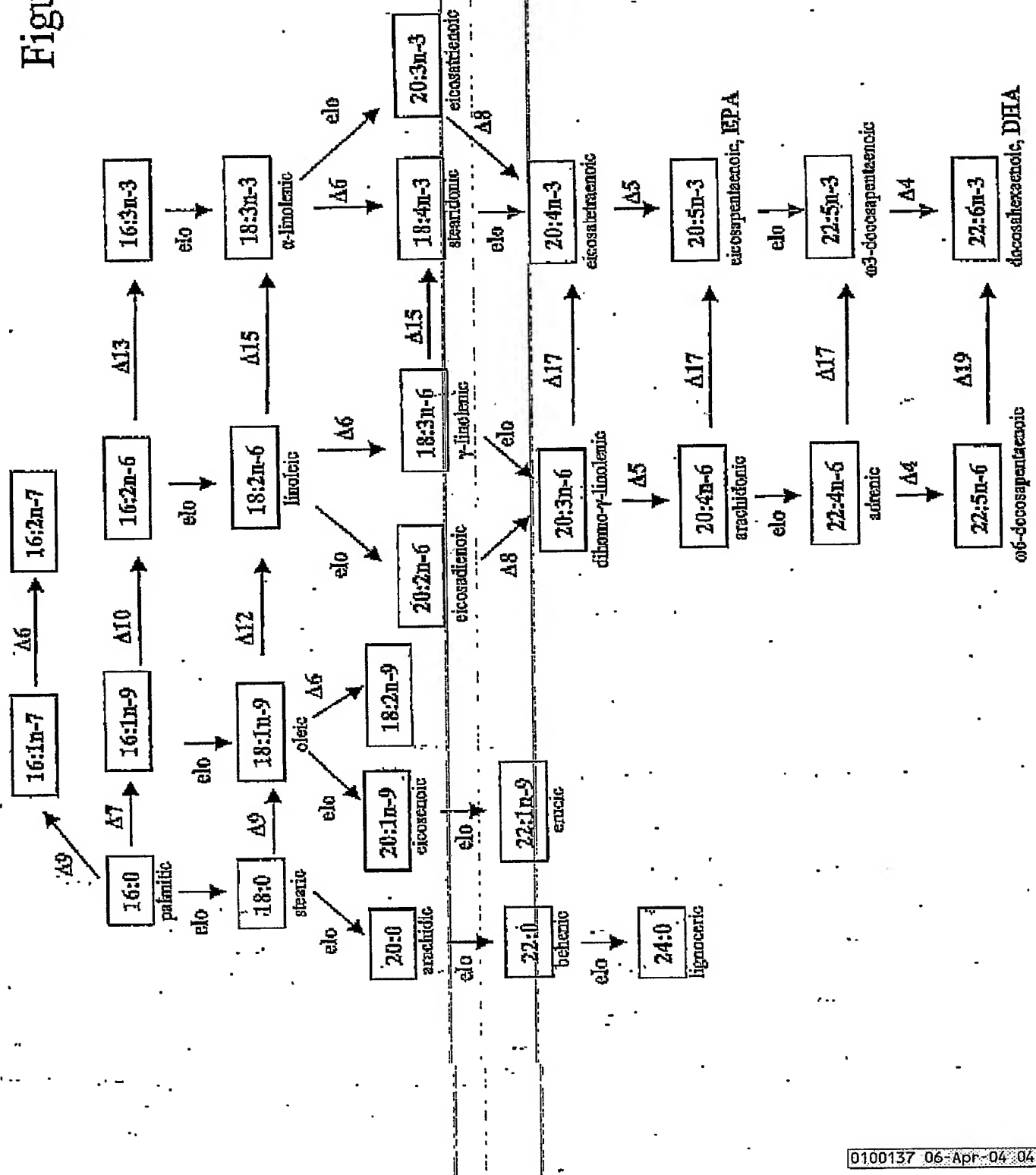


Figure 14

TpdesO expression in Yeast. Results shown are from samples after 6 days culture in presence of exogenously fed fatty acids

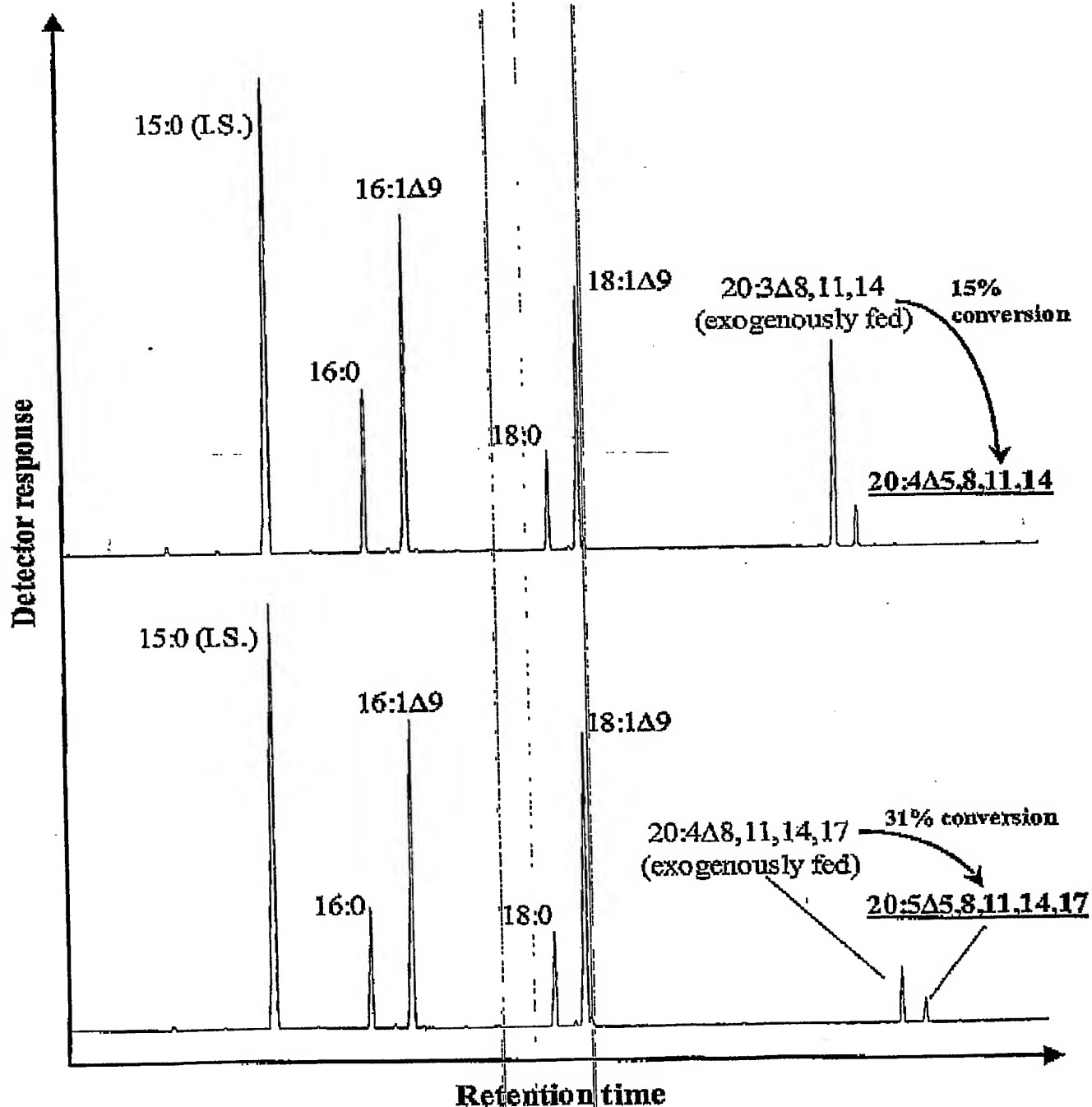


Figure 12c

